EFFECTS OF HYDROGEN PEROXIDE/UV ADVANCED OXIDATION PROCESS SANITIZATION ON DUCK AND TURKEY EGG MICROBIAL LOADS AND HATCHABILITY

A Thesis

by

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ABSTRACT

Commercial duck and turkey hatching eggs typically have high microbial loads due to their highly soiled nature. As a result, the commercial turkey and duck breeder industries typically wash hatching eggs prior to incubation. Previous studies with chicken eggs have determined that egg sanitization with the combination of hydrogen peroxide and ultraviolet light in an advanced oxidation process (H_2O_2/UV AOP) decreases eggshell surface microbial loads and may result in improved hatchability. The objective of this study was to evaluate the effectiveness of the H_2O_2/UV AOP method of egg sanitization on turkey and duck eggshell microbial loads, hatchability, and overall hatchling quality. Additionally, the effectiveness of the H_2O_2/UV AOP method was evaluated in a commercial setting alone or in combination with the standard washing process for duck eggshell microbial load reductions.

Microbiological evaluations determined that turkey and duck eggs treated with the H_2O_2/UV AOP method resulted in reduction of surface and subsurface eggshell aerobic plate counts (APC) compared to untreated eggs. In a commercial setting, the combination of the washing process followed by the H_2O_2/UV AOP method resulted in lower eggshell APC compared to the washing process alone and the H_2O_2/UV AOP method alone.

Treatment of duck eggs with the H_2O_2/UV AOP method resulted in an improvement in hatchability by 13% compared to untreated eggs and 9% compared to the washing process. Additionally, duckling quality results indicated an increase in percentage of ducklings without quality defects for the washed and the H_2O_2/UV AOP treated compared to untreated control. However, treatment of turkey eggs with the H_2O_2/UV AOP method had no impact on hatchability or poult quality. Results obtained in this study suggest that utilization of the H_2O_2/UV AOP method of egg sanitization could have important economic implications to the

duck breeder industry. Additional research is warranted to evaluate the effects of using the H_2O_2/UV AOP method in a commercial setting for microbial load reductions, hatchability and hatchling quality for turkey and duck eggs.

DEDICATION

This thesis is dedicated to my mother and grandmother, Delia Gutierrez and Nora Leal. You are the strongest and most admirable women I have ever known. Thank you for all your love and support. I could never thank you enough for all the sacrifices you had to make in order for me to succeed. Because of you, this was possible.

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NOMENCLATURE

AOP	advanced oxidative process
APC	aerobic plate count
cfu	colony forming unit
d	day
h	hour
H_2O_2	hydrogen peroxide
HU	Haugh unit
LOD	limit of detection
log	logarithmic
min	minute(s)
mL	milliliter
PBS	phosphate buffered saline
QAC	quaternary ammonium compounds
RO	reverse osmosis
ROS	reactive oxygen species
UV	ultraviolet light

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CHAPTER I

INTRODUCTION

Commercial duck and turkey hatching eggs typically have high microbial loads due to their highly soiled nature. Thus, duck and turkey hatching eggs are commonly washed in commercial settings (Patterson, et al., 1990). The egg washing process commonly involves the use of a commercial egg washing machine followed by the application of a sanitizing agent such as quaternary ammonium compounds (QAC), hypochlorite solutions, phenolic compounds, or various antibiotic solutions (Patterson, et al., 1990). Eggshell microbial contamination can have negative effects on hatchability and hatchling quality. If eggshells are contaminated with pathogens, these organisms could follow the flock to the grow-out farm, and could lead to food safety hazards in the final products (Berrang, 1999; Coufal, et al., 2003).

Previous studies have indicated that the use of hydrogen peroxide (H_2O_2) and ultraviolet light (UV) in combination in an advanced oxidation process (AOP) is an effective method for egg sanitization. The H_2O_2/UV AOP method has been proven to be highly effective for reducing eggshell microbial loads on chicken eggs (Wells, et al., 2011b; Fuchs, 2013; Al-Ajeeli, et al., 2016; Rehkopf, et al., 2017) and improving hatchability (Fuchs, 2013). An AOP is an aqueous phase process that causes oxidation and leads to inactivation of microbial cells through the action of hydroxyl radicals (Legrini, et al., 1993; Comninellis, et al., 2008). Photolysis of the peroxide bond in H_2O_2 yields hydroxyl radicals that have an unpaired electron that easily interacts with vital cellular components such as lipids, proteins, DNA, and carbohydrates to ultimately cause cell inactivation (Shimoda, et al., 1997; Mamane, et al., 2007; Ikai, et al., 2010).

Hatching egg sanitization is an essential preventative step for reducing microbial contamination in poultry production (Spickler, et al., 2011). This is particularly true in antibiotic-

free production. Implementing an effective and commercially feasible method of egg sanitization could be beneficial by increasing flock survivability, and thus increasing economic gain (Sheldon and Brake, 1991; Berrang, et al., 1997).

The effects of the H_2O_2/UV AOP method of egg sanitization has not been previously researched on turkey and duck hatching eggs. The primary objective of this study was to evaluate the effects of the H_2O_2/UV AOP as a method of egg sanitization on turkey and duck hatching eggs. More specifically, data was collected to determine the effects on eggshell microbial loads, hatchability, embryonic mortality, egg moisture loss, and hatchling quality. In addition, it was of interest to compare the effects of the H_2O_2/UV AOP method of egg sanitization to the egg washing method currently used on turkey and duck eggs in commercial operations.

CHAPTER II

LITERATURE REVIEW

Innate egg defenses

Avian eggs possess innate defense mechanisms against microbial invasion for the protection of the developing embryo. These defenses consist of the cuticle or "bloom", the shell, the inner and outer shell membranes, and the albumen (Brown, et al., 1965; Berrang, 1999; Li, et al., 2016; Sylte, et al., 2017). The cuticle is a thin layer on the eggshell surface that provides coverage to most pores of the eggshell (decreasing eggshell porosity), thus, serving as the first physical and chemical line of defense against microbial invasion and excessive loss of moisture (Sheldon and Brake, 1991; Berrang, 1999; Solomon, 2010; Sylte, et al., 2017). It is composed of proteins, carbohydrates and lipids (Yamamoto, et al., 1997). Previous studies suggested that the removal or alteration of the cuticle led to approximately 40% increase in microbial contamination, excessive moisture loss during incubation, and in turn, an embryonic mortality increase (Sander and Wilson, 1999; Fuchs, 2013; Stepinska, et al., 2017). Thus, there is disagreement and hesitation among poultry producers and scientists about the use of egg sanitization on hatching eggs due to the concern regarding cuticle integrity (Patterson, et al., 1990; Sander and Wilson, 1999; Coufal, et al., 2003). The shell provides physical protection against microbial invasion. Shell thickness, quality (deformities or visible cracks), and porosity have been linked to influence microbial contamination of the egg. Additionally, increased eggshell contact with microorganisms directly influences the capacity of microbial invasion (Board and Fuller, 1974; Yamamoto, et al., 1997). Conversely, scientists have argued that shell thickness does not significantly impact bacterial penetration, but the coverage of the cuticle and its ability to decrease porosity is more important (Berrang, 1999).

Apart from the cuticle and shell, the inner and outer shell membranes also provide protection against microbial invasion. The shell membranes behave as filters and temporary barriers against microorganisms. Their structure consists of keratin layers encapsulated in glycoprotein. Although effective against the inward movement of bacteria, the inner and outer shell membrane barrier structures are not effective against hyphae of molds (Board and Fuller, 1974). Previous data suggested the inner shell membrane is more effective than the outer membrane for prevention against microbial contamination of the egg's internal contents due to its tighter meshwork structure (Berrang, 1999).

The albumen also plays an important role as a natural defense mechanism. Its viscous consistency delays the inward movement of microorganism toward the egg yolk. Additionally, it provides an unfavorable environment for microorganisms due to its alkaline pH and inhibitory proteins (Brown, et al., 1965; Berrang, 1999; Wellman-Labadie, et al., 2008; Sylte, et al., 2017). The albumen pH has been shown to have bactericidal activity at a pH of 9, and bacteriostatic activity at a pH of 7 (Seviour and Board, 1972; Wellman-Labadie, et al., 2008). In chicken eggs, the albumen is composed of approximately 10% protein. The proteins of the albumen include ovalbumin, conalbumin (ovotransferrin), ovomucoid, lysozyme, ovomucin, ovoglobulin, ovomacroglobulin, ovoglycoprotein, flavoprotein, ovoinhibitor, cystatin, and avidin (Yamamoto, et al., 1997).

The most prominent albumen protein is ovalbumin, representing over half the total albumen protein (54%). However, there is little evidence of any antimicrobial properties of ovalbumin. Conalbumin, ovomucoid, and lysozyme have demonstrated to contribute effective defense against microbial growth and invasion of the developing embryo (Yamamoto, et al., 1997). Conalbumin constitutes approximately 12% of the total albumen protein and has similar

function as lactotransferrin. Conalbumin is a single polypeptide protein that is able to bind free iron. Previous research has indicated that bacteria are unable to grow in the presence of conalbumin unless conalbumin is quenched with iron. This defense is especially important against Gram-negative bacteria (Board and Fuller, 1974; Yamamoto, et al., 1997). Ovomucoid is about 11% of the total albumen protein. It is a trypsin inhibitor glycoprotein that functions best for inhibition of bacterial and fungal proteinases (Yamamoto, et al., 1997).

Lysozyme constitutes approximately 3.5% of the total albumen protein. It induces osmotic lysis by hydrolyzing glycosidic bonds in peptidoglycan cell walls of Gram-positive bacteria. Lysozyme is also capable of inactivating Gram-negative bacteria by interacting with their outer membrane lipopolysaccharides. Furthermore, lysozyme is also able to increase the viscosity of the albumen by interacting with ovomucin (Board and Fuller, 1974; Yamamoto, et al., 1997). However, previous research has emphasized that lysozyme plays a minor role in antimicrobial (chemical) defense, and mostly functions as a physical defense against microbial infection (Board and Fuller, 1974).

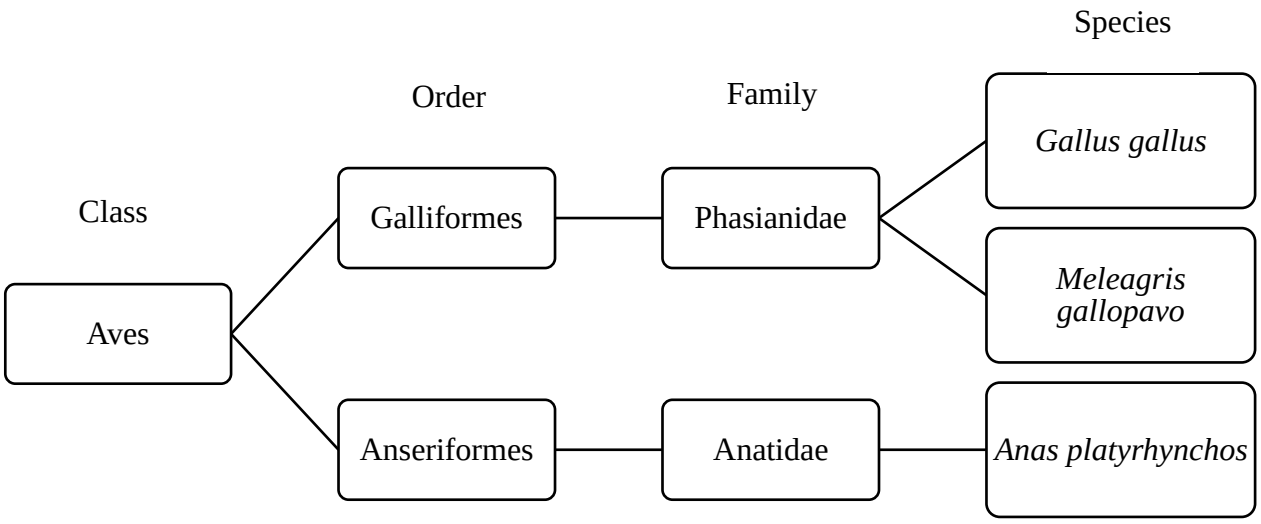
Differences between domestic chicken, turkey, and duck species

Poultry production has been rapidly increasing due to consumer demand for high-quality products at a lower cost (Scanes, 2007). The commercial poultry industry has been able to increase production to meet exponential world-wide consumption rates due to improvements in technology, nutrition, and genetics. In 2005, over 82 million tons of poultry meat was produced worldwide (Scanes, 2007). Out of the poultry meat produced in 2005, chicken meat was the most produced at 71.8 million tons, turkey meat was 5.2 million tons, and duck meat was 3.5 million tons (Scanes, 2007). It is important to understand the differences between chickens, turkeys, and

ducks in order to make advancements in more efficient methods of production that suit each individual species.

Phylogenetically, domestic duck species (*Anas platyrhynchos*) differ in family type from domestic chicken (*Gallus gallus*) and turkey (*Meleagris gallopavo*) species. Domestic chicken and turkey species differ in subfamily type as depicted in Figure 1 (Couch and Saloma, 1973; Zhang, et al., 2014). Apart from differences in taxonomic classification, chickens, turkeys, and ducks differ in their egg properties (Table 1). Differences in egg weight, shell membrane relative density, and albumen components could have an impact on egg susceptibility to microbial contamination. A study conducted by Brown et al. (1965) compared the structures of chicken, turkey, and duck eggs and their bacterial susceptibility when eggs were fresh and stored (microbial invasion over time). The study demonstrated that chicken shell membranes had the highest relative density and turkey shell membranes had the lowest. That study also indicated thickness differences between the species' inner shell membranes. Chicken inner shell membranes were the thickest, and duck inner shell membranes were the thinnest. Microbial susceptibility results obtained in that study demonstrated that fresh duck eggs were the most susceptible to contamination when compared to both fresh chicken and turkey eggs. This further emphasizes the importance of the inner shell membrane as an innate defense mechanism against microbial penetration of the egg.

Figure 1. Phylogenetic tree of domestic chicken, turkey and duck species[1]



[1]Adapted from Couch and Saloma (1973); Zhang, et al. (2014).

Table 1. Properties of chicken, turkey, and duck eggs that might influence the potential of microbial invasion of the egg

Egg properties	Chicken	Turkey	Duck
Average egg weight (g)[1,2,3,4,5]	56	87	80
% Shell[1,2,4,6,7]	9	10	12
% Yolk[2,4,6,7]	31	31	32
% Albumen[2,4,6,7]	59	57	50
Energy (kcal/g)[2]	1.65	1.65	1.75
Inner shell membrane weight (g)[8]	0.03	0.05	0.03
Outer shell membrane weight (g)[8]	0.15	0.27	0.23
Inner shell membrane thickness (µm)[8]	6.58	5.30	4.41
Outer shell membrane thickness (µm)[8]	15.04	29.77	26.12
Relative density of shell membranes[8,14]	2.56	1.02	1.22
Albumen pH (Fresh)[4,9,10,11,12,]	8.43	8.45	8.52
Albumen to yolk ratio[3]	1.95	1.27	1.34
Haugh unit[5,9,13]	75.00	77.13	74.40

[1]Asmundson and Baker (1940).
[2]Ricklefs (1977).
[3]Nisianakis, et al. (2009).
[4]Hristakieva, et al. (2011).
[5]Popoola, et al. (2015).
[6]Asmundson (1939).
[7]Asmundson, et al. (1943).
[8]Brown, et al. (1965).
[9]Juárez-Caratachea, et al. (2011).
[10]OnbaŞılar, et al. (2011).
[11]Dorji (2014).
[12]Ipek and Sozcu (2017).
[13]Yenice, et al. (2016).
[14]Relative density was calculated as the ratio of the outer to inner shell membrane weight divided by the ratio of the outer to inner shell membrane thickness (Brown, et al., 1965).

As previously noted, the albumen is an important defense mechanism against microbial invasion. On average, chicken eggs contain a higher percentage of albumen, and have the highest albumen to yolk ratio when compared to turkey and duck albumen. Additionally, Haugh units between the species vary, with duck eggs having the lowest and turkey eggs having the highest Haugh unit measurements. Although Haugh unit measurements are an accurate method to evaluate albumen protein quality, it is not an accurate indicator of antimicrobial protein activity

between the species. A study conducted by Wellman-Labadie, et al. (2008) evaluated enzymatic content of lysozyme in chicken, goose, swan, duck, and merganser eggs. They determined that duck species had the highest lysozyme content when compared to the other waterfowl species. However, compared to chicken lysozyme content, waterfowl have less lysozyme content. Their study also indicated that there were no differences in conalbumin concentration or antimicrobial activity between the species. Furthermore, it was previously noted by Board and Fuller (1974) that the concentration of lysozyme varies with species, but did not indicate differences of other antimicrobial albumen proteins. Additional research should be conducted to determine concentrations of antimicrobial proteins in the albumen such as conalbumin and ovomucoid in different poultry species. Evaluation of the concentration and antimicrobial activity of these proteins would further help determine if differences exist in microbial susceptibility between chicken, turkey, and duck species.

Microbial contamination of eggs

Eggshell and internal egg content contamination occurs in poultry eggs in spite of multiple egg defense mechanisms. Contamination by potential pathogens could occur through vertical transmission from pre-existing infection of the hen. More commonly, horizontal transmission is due to potential pathogens present in feces, feathers, nest boxes, storage rooms, transportation vehicles, equipment, and other environmental sources (Barbour, et al., 1985; Chavez, et al., 2002; Wells, et al., 2010). Assuming the egg is sterile during formation, microorganisms could penetrate the eggshell during the first 30 to 60 sec after oviposition (prior to hardening of the cuticle) via an eggshell contraction (Sacco, et al., 1988; Berrang, et al., 1997; Berrang, 1999; Coufal, et al., 2003). The temperature change from the hen's internal temperature to the outside environment during lay leads to an increase in negative egg pressure which causes

a contraction of the eggshell. During this contraction, microorganisms and water surrounding the egg could be internalized into the eggshell and its membranes (Berrang, et al., 1997; Coufal, et al., 2003). This contraction could also occur when eggs are transferred from an egg storage cooler. In this scenario, the moisture in the air causes condensation on the egg, thus helping to mobilize eggshell microorganisms and facilitate eggshell penetration (Berrang, 1999). Contamination could also occur during embryonic development. Poultry hatcheries have been found to be highly contaminated with various microorganisms that spread easily by employee activity, air ventilation, and improper sanitization techniques of incubators and hatchers. (Sheldon and Brake, 1991; Berrang, et al., 1997; Sander and Wilson, 1999).

Pathogenic contamination of the egg could result in embryonic mortality, low hatchability, poor hatchling quality, and loss of chick performance. Successful pathogenic contamination of the egg could also result in the pathogen colonizing the embryo's intestinal tract, thus, following the flock to the grow-out farm to further spread the infection and cause food safety hazards (Berrang, 1999; Wells, et al., 2011a; Sylte, et al., 2017). This contamination can be caused by active (fungi) or passive (bacteria) translocation. Previous studies have demonstrated that most of the eggshell's surface is contaminated with Gram-positive bacteria such as *Enterococcus* spp. and *Staphylococcus* spp. The pathogenic bacteria that are most prominent to cause rotting during storage and incubation are Gram-negative bacteria such as *E. coli* and *Pseudomonas* spp. (Board and Fuller, 1994).

Pathogens of concern

Apart from *Salmonella, E. coli* is one the primary pathogens of concern associated with the poultry industry (Montgomery, et al., 1999). *Escherichia coli* is a Gram-negative, rod-shaped bacteria that is naturally occurring in the lower digestive tract of mammals and birds (Bauman,

2014). Over time, *E. coli* infections have become a growing concern due to bacterium mutation and antibiotic resistance. *E. coli* infection in chickens may cause airsacculitis, arthritis, enteritis, cellulitis, and other secondary infections (Montgomery, et al., 1999). It is also known that *E. coli* isolates have the ability to cause embryonic mortality. Montgomery, et al. (1999) conducted a study to evaluate the effects of *E. coli* on the developing chicken embryo and the grow-out phase. The study demonstrated that eggs inoculated with *E. coli* hatched very poorly when compared to the control eggs (non-inoculated). The chicks from inoculated eggs that were able to survive the *E. coli* infection had lower chick quality and had a slower rate of yolk absorption than the chicks from non-inoculated eggs. In the grow-out phase, the chickens from *E. coli* infected eggs had higher early mortality and lower weight gain than the control. That study also indicated that *E. coli* was able to not only survive, but also multiply in the developing embryo. *Escherichia coli* was inoculated at a concentration of 10^1 to 10^2 organisms per egg on day 12 of embryogenesis, and after 7 days post-inoculation the concentration increased to 10^3 organisms per egg (Montgomery, et al., 1999). This is evidence that pathogenic strains of *E. coli* are able to surpass the natural defenses of the egg, multiply during incubation, and ultimately infect chickens in the grow-out farm.

Enterococcus spp. are also known to cause infections in poultry (Dutta and Devriese, 1982). *Enterococcus* spp. are Gram-positive, coccoid bacteria that may occur in short chains, or singularly (Bauman, 2014). In poultry, *Enterococcus* infection may cause septicemia, joint infections, cellulitis, osteomyelitis, endocarditis, and other secondary infections. These bacteria are easily transmitted via oral ingestion, inhalation of aerosols, or through skin injuries. Typically, infections by this bacterial species are able to be controlled by the use of antibiotics

(Morishita, 2018). However the demand for antibiotic-free poultry will likely increase the occurrence of *Enterococcus* infection in the commercial industry.

Staphylococcus aureus is a Gram-positive, coccoid bacteria (Bauman, 2014) that is a normal resident of the skin, feathers, respiratory, and intestinal tract of poultry. These bacteria are of growing concern in the poultry industry due to its high morbidity, and mortality to poultry, and ability to cause foodborne illness in humans. In poultry, *Staphylococcus aureus* may cause mild skin infections such as tenosynovitis, gangrenous dermatitis, impetigo, and life threatening diseases such as pneumonia, septicemia, and toxic shock syndrome (Shareef, et al., 2009; Li, et al., 2016). As with other infectious bacteria, antibiotics are currently used to control *S. aureus* infections. However, as mentioned previously, the poultry industry is moving towards antibiotic-free production, which will likely increase the incidence of bacterial infections in the future. A study conducted by Shareef, et al. (2009) demonstrated that *S. aureus* was highly prominent on the laying hen's feet (87.5%), feeders and drinkers (83.3%), chicken eggshells (16.6%), hatchery and working surfaces (75%), and in day old chicks (29.1%).

Yeast and molds are also known to cause disease and embryonic mortality in poultry. Yeast and molds are fungi that are single-cellular and multicellular microorganisms, respectively (Bauman, 2014). Fungi infections in poultry may cause mycoses and mycotoxicoses (Szablewski, et al., 2010). The survivability of micro-fungi greatly depends on the environmental conditions in which they reside (Board and Fuller, 1974). However, it is known that various micro-fungal species can survive in harsh environments. Moreover, certain species of micro-fungi are capable of digesting the cuticle of the egg, and are able to more readily penetrate the pores and shell membranes of the egg (Board and Fuller, 1994).

Egg disinfecting methods

Common egg disinfectants used in the poultry industry include: chlorine products, quaternary ammonium compounds (QAC), hydrogen peroxide (H_2O_2), and ultraviolet (UV) radiation. Chlorine is commonly used as a final egg washing step in the commercial production of eggshells. Patterson, et al. (1990) performed a study to evaluate the effects of chlorine foam as a method of duck egg sanitization on surface eggshell microbial loads and hatchability. The study indicated that the use of chlorine foam was effective at reducing eggshell microbial loads on inoculated duck eggs, and demonstrated an 11% hatchability improvement of highly soiled duck eggs when compared to eggs that were left untreated.

Quaternary ammonium compounds are also commonly used as an egg disinfectant for both shell and hatching eggs. A study conducted by Arhienbuwa, et al. (1980) on the effects of QAC as a method of egg sanitization on turkey hatching eggs demonstrated a significant reduction of microbial loads on eggshell surfaces when compared to eggs that were not treated. Limited research has been conducted on the effects of QAC as a turkey hatching egg disinfectant on hatchability. A study conducted by Sacco et al. (1989) on the effects of QAC on turkey hatching eggs demonstrated no differences in eggshell microbial loads and no improvement in hatchability when compared to eggs treated with formaldehyde fumigation. However, that study only compared the effects of QAC to formaldehyde fumigation or to QAC plus formalin (37% formaldehyde solution) and did not include a non-sanitized control.

The use of H_2O_2 as a method of hatching egg sanitization has also been investigated to determine its impacts on microbial loads and hatchability. Hydrogen peroxide is a commonly used sanitizing liquid that is colorless, odorless, and safe to use when handled properly. Hydrogen peroxide degrades into water and oxygen leaving no toxic residues (Sheldon and

Brake, 1991; Sander and Wilson, 1999). Previous studies using chicken eggs demonstrated that egg sanitization using H_2O_2 reduced eggshell microbial loads by approximately 1 log_{10}cfu/egg without negative impact to hatchability. studies (Sheldon and Brake, 1991; Spickler, et al., 2011).

The effects of UV radiation has also been studied on hatching eggs for microbial load reductions. UV radiation can induce microbial inactivation by depolarization of cellular membranes (and increase in cell membrane permeability), alteration of DNA, RNA, and protein structures, and inhibition of oxidative phosphorylation (Kuo, et al., 1996; Mamane, et al., 2007). Previous research has indicated that egg irradiation by UV is an effective method to decrease microbial loads on eggshell surfaces on both chicken (Kuo, et al., 1996; Chavez, et al., 2002) and turkey (Russo, 2001) hatching eggs without negative impacts to hatchability. A study conducted by Russo (2001) using UV to irradiate turkey hatching eggs reduced the amounts of *E. coli* and *Salmonella* on the surface of eggshells when compared to untreated eggs. Embryonic mortality, bird weight, and hatchability of UV irradiated eggs were not negatively impacted.

The H_2O_2/UV AOP method

The first study that evaluated the effects of the H_2O_2/UV AOP as a chicken egg sanitization method on surface microbial loads was conducted by Wells, et al. (2010). That study evaluated different concentrations of H_2O_2 and length of UV exposure with the ultimate goal to determine the optimum combination that would yield greater eggshell microbial load reductions. They determined that the optimal combination was 1.5% H_2O_2 and 8 min of UV radiation of eggs. To further study the H_2O_2/UV AOP as a chicken egg sanitization method, two studies were conducted to evaluate its effects on surface eggshell microbial loads and hatchability (Wells, et al., 2011a; Wells, et al., 2011b). Those studies determined that the H_2O_2/UV AOP method on

chicken eggs resulted in reduction of surface microbial loads; however, there was no improvement in hatchability.

The H_2O_2/UV AOP is an effective antimicrobial process due to cell inactivation via hydroxyl radical interactions with vital cellular components. An AOP is an oxidative reaction in which photocatalysis of the peroxide bond in H_2O_2 produces hydroxyl radicals (Legrini, et al., 1993; Mamane, et al., 2007; Comninellis, et al., 2008). Hydrogen peroxide is considered to be a weak reactive oxygen species (ROS) but holds potential for greater microbial inactivation when combined with ultraviolet light, electrolysis, ozonation, Fenton's reagent, or ultrasound (Chapple and Matthews, 2007; Comninellis, et al., 2008). However, in the poultry industry it is important to preserve the structure and functionality of the innate antimicrobial egg defenses, especially the cuticle. Ultraviolet light irradiation of chicken hatching eggs has indicated to decrease eggshell microbial loads without compromising the cuticle (Coufal, et al., 2003).

Hydroxyl radicals are a highly potent, short-lived, reactive oxygen species (ROS) which can easily diffuse into the eggshell inner and outer membranes (Clifford and Repine, 1982; Legrini, et al., 1993; Sander and Wilson, 1999). Hydroxyl radicals target vital cellular and extracellular components. Intracellular components that may be damaged include lipids via peroxidation, carbohydrates, proteins via oxidation of aliphatic amino acids, DNA and RNA via hydrogen atom abstraction, antiproteases via oxidation, and light molecular weight species (glutathione). Extracellular components that may be damaged include the extracellular matrix components (proteoglycan) and the collagens and structural proteins (Badwey and Karnovsky, 1980; Chapple and Matthews, 2007; Mamane, et al., 2007).

A study conducted by Al-Ajeeli, et al. (2016) compared chlorine, QAC, paracetic acid, paracetic acid in combination with UV, and H_2O_2 in combination with UV (H_2O_2/UV AOP)

sanitizing methods on chicken eggs. That study determined that the H_2O_2/UV AOP method of egg sanitization was the most effective at reducing microbial loads on the surface of the eggshell. A more recent study conducted by Rehkopf, et al. (2017) evaluated the effectiveness of H_2O_2/UV AOP method of chicken egg sanitization for *Salmonella* reductions. That study demonstrated that the H_2O_2/UV AOP method of egg sanitization resulted in significant decrease of *Salmonella* on chicken eggs. Additionally, a study conducted by Fuchs (2013) on the effects of the H_2O_2/UV AOP on chicken hatching eggs evaluating microbial loads, hatchability, and overall chick quality has also been conducted. That study demonstrated that the H_2O_2/UV AOP method was effective at reducing eggshell microbial loads and increasing hatchability without negative impacts to chick quality.

Numerous research studies have been conducted to evaluate the effects of the H_2O_2/UV AOP method of egg sanitization on chicken eggs. However, there has been no previous research regarding the effects of the H_2O_2/UV AOP method of egg sanitization on turkey and duck hatching eggs. Since turkey and duck eggs are known to be highly soiled in nature and contain high microbial counts, an effective method of egg sanitization could have significant implications for hatchability, hatchling quality, and potentially reducing pathogenic microorganisms that might cause disease outbreaks in the grow-out phase and possibly result in food safety concerns for consumers. Therefore, the goals of the present study to evaluate the effectiveness of the H_2O_2/UV AOP method as an egg sanitization method on turkey and duck hatching eggs are novel and warranted.

CHAPTER III

EFFECTIVENESS OF DUCK HATCHING EGG SANITIZATION WITH THE H_2O_2/UV ADVANCED OXIDATIVE PROCESS

Introduction

Limited research has evaluated hatching egg sanitization methods and their effectiveness on duck eggs. Compared to chicken eggs, duck egg hatch of fertile is commonly lower due to the high number of soiled eggs (Patterson, et al., 1990). Bacteria present in the environment can penetrate the eggshell via contraction of the egg contents that occurs during egg cooling. This occurs after the laying process and also after eggs are placed in a storage cooler (Berrang, 1999; Wells, et al., 2010). Eggs contain natural barriers against bacterial penetration such as the cuticle or "bloom", the tight meshwork of the inner shell membrane, the high pH of the albumen, and the presence of inhibitory proteins in the albumen (Berrang, 1999). However, microbial contamination inside hatching eggs is not uncommon and can affect hatchability, hatchling quality, and follow a flock to the grow-out farm and cause disease (Berrang, 1999). Therefore, duck eggs are typically washed in commercial breeder operations to remove adhering organic material and sanitized with disinfecting agents such as quaternary ammonium products, hypochlorite solutions, phenolic compounds, or various antibiotic solutions (Patterson, et al., 1990).

Previous studies have indicated that using a combination of hydrogen peroxide and ultraviolet light (H_2O_2/UV) as a method of egg sanitization is highly effective for reducing eggshell microbial loads (Wells, et al., 2010; Wells, et al., 2011b; Al-Ajeeli, et al., 2016; Rehkopf, et al., 2017). The combined application of H_2O_2 and UV forms an advanced oxidation reaction (AOP) that is highly antimicrobial. An AOP is an aqueous phase process that causes

oxidation and leads to inactivation of pathogenic cells through the action of hydroxyl radicals (Legrini, et al., 1993; Comninellis, et al., 2008). Photolysis of the peroxide bond in H_2O_2 yields hydroxyl radicals that have an unpaired electron that easily interacts with vital cellular components such as lipids, proteins, DNA, and carbohydrates to ultimately cause cell death (Shimoda, et al., 1997; Mamane, et al., 2007; Ikai, et al., 2010).

Using the H_2O_2/UV AOP as an egg sanitization method on chicken hatching eggs has resulted in increased hatchability without negative effects on embryonic mortality or chick quality parameters when compared to untreated eggs (Wells, et al., 2011b; Fuchs, 2013). The objective of this experiment was to compare the use of the H_2O_2/UV AOP method of egg sanitization to a conventional commercial egg washing method and evaluate duck egg microbial loads, hatchability, and duckling quality.

Materials and methods

Eggs and treatments

Pekin duck hatching eggs were obtained from the same commercial duck breeder company for all 3 trials conducted in this experiment. Two groups of eggs were simultaneously shipped directly from the duck breeding company to Texas A&M University for each trial. The first group consisted of eggs that were washed at a commercial duck hatchery using a combination of water, disinfecting agents, and an anti-foaming ingredient. The second group consisted of eggs that did not undergo any form of washing or sanitization prior to shipment. Upon arrival, these eggs were randomly divided into two treatments. Half of the untreated eggs remained untreated to serve as the control group. The other half of the untreated eggs were sanitized with the H_2O_2/UV AOP prototype egg sanitizer as described in Al-Ajeeli, et al. (2016). Hydrogen peroxide and reverse osmosis (RO) water were pre-heated in an incubator set at 37°C and

combined to yield 3% H_2O_2 concentration prior to egg treatment. The H_2O_2/UV AOP egg sanitization mechanism consisted of two repetitions of the combination of 3% H_2O_2 spray followed by immediate UV light exposure. Upon exiting the sanitizer, the eggs were allowed to sit for approximately 3 min prior to conveying them through the sanitizer a second time to assist in removal of adhering organic material. Thus, eggs in the sanitized group were exposed to 4 total combinations of H_2O_2 and UV treatment.

Microbial analysis

Ten eggs were randomly selected per treatment per trial to evaluate eggshell microbial loads. Eggs were sampled using tongs that were sterilized by dipping them in 100% ethanol followed by flaming. Sampled eggs were placed in Whirl-Pak bags (Nasco, Fort Atkinson, WI) with 20 mL of sterile PBS (pH 7.4; HiMedia Laboratories, West Chester, PA). Eggs were massaged by hand for 1 min in the bag. Ten-fold serial dilutions were performed, and 1 mL of each egg rinse solution and dilution was plated onto aerobic plate count (APC) Petrifilms (3M United States, Maplewood, MN). After 48 h of incubation at 37°C, colonies were enumerated and total APC were calculated as $\log_{10}$cfu/egg. Therefore, the limit of detection for this assay was 20 cfu/egg, or 1.3 $\log_{10}$cfu/egg. A value of 1.0 $\log_{10}$cfu/egg was assigned to eggs with zero counts.

Incubation, hatching, and moisture loss

Three incubators (Model 1500 GQF incubators, GQF Manufacturing Company Inc., Savannah, GA) with 3 paired hatchers (Model 1550 GQF hatchers, GQF Manufacturing Company Inc., Savannah, GA) were utilized per treatment per trial. Incubators and hatchers were randomly rotated among treatments between trials to account for incubator effect. Approximately 198 eggs were placed per machine per trial, with some variation depending on the number of

eggs damaged during transport and handling. The assignment of each incubator (and paired hatcher) and the corresponding number of eggs set per treatment per trial are presented in Table 2.

Table 2. Total duck eggs placed in incubators per treatment per trial

Trial	Treatment	Incubator number	Number of eggs[1]
1	Control	2, 6, 8	198,198,198
	Washed	1, 4, 9	198,198,198
	Sanitized	3, 5, 7	198,198,198
2	Control	3, 5, 7	163,198,198
	Washed	2, 6, 8	198,198,198
	Sanitized	1, 4, 9	198,198,198
3	Control	1, 4, 9	198,198,157
	Washed	3, 5, 7	198,192,196
	Sanitized	2, 6, 8	198,198,198
Total	Control		1,706
	Washed		1,774
	Sanitized		1,782

[1]Corresponds with incubator number, respectively.

Duck eggs were incubated at 37.5°C and 55 to 60% relative humidity for 25 d. On day 26, eggs were transferred to hatchers set at 36.9°C and 65 to 70% relative humidity. On day 28 of incubation, hatched ducklings were counted and recorded per incubator. Ducklings were weighed in trays to assess average hatch weight. The remaining unhatched eggs were broken out and classified as infertile, early dead (0 d to 14 d), late dead (15 d to 28 d), pipped, or rotten. Lastly, all hatched ducklings were examined for quality issues such as naval tags, leg problems, dirty feathers, or cull birds due to other visible deformities.

Egg percent moisture loss during incubation was evaluated in Trial 3 only. Prior to setting the eggs in the incubators for Trial 3, empty egg flats were numbered and weighed. Flats of duck were weighed prior to incubation, and then again during transfer to hatchers on day 26.

Egg moisture loss was then calculated as a percentage of the weight of the eggs prior to incubation.

Statistical analysis

A GLM using y=treatment, trial, and treatment by trial was utilized. Assumptions of equal variance and normality were met for ANOVA with means separated by LSD post hoc test. Mean differences were considered significant at $P < 0.05$.

Results and discussion

Eggshell microbial counts

All trials in this experiment indicated similar results for eggshell surface microbial loads with no significant differences observed for treatments between trials (Table 3). When comparing treatments, the control eggs had the greatest APC compared to the washed ($P < 0.001$) and sanitized ($P < 0.001$) eggs. The average APC over the 3 trials for control, washed, and sanitized eggs (5.82, 2.27, and 2.31 $\log_{10}$ cfu/egg, respectively). The data observed in this study demonstrated that the sanitized treatment had similar microbial load reductions to the commercial duck egg washing method. These results are similar to previous studies that tested the effects of the H_2O_2/UV AOP method on chicken eggs where APC and *Salmonella* were reduced when compared to untreated eggs (Wells, et al., 2011b; Fuchs, 2013; Al-Ajeeli, et al., 2016; Rehkopf, et al., 2017). It is important to decrease initial eggshell microbial loads prior to incubation. Excessive eggshell microbial contamination has been associated to increased embryonic mortality and decreased hatchability (Sacco, et al., 1988).

Table 3. Duck eggshell surface aerobic plate counts (log_{10} cfu/ml ± SE) for trials 1, 2 and 3[1]

Treatment	Trial 1	Trial 2	Trial 3	Average
Control	6.32 ± 0.10^{a}	5.57 ± 0.09^{a}	5.57 ± 0.09^{a}	5.82 ± 0.08^{a}
Washed	2.50 ± 0.38^{b}	2.35 ± 0.33^{b}	1.96 ± 0.15^{b}	2.27 ± 0.18^{b}
Sanitized	2.49 ± 0.49^{b}	1.94 ± 0.37^{b}	2.49 ± 0.32^{b}	2.31 ± 0.23^{b}

[a,b]Means within a column having different superscripts are significantly different ($P < 0.05$).
[1]n = 10 eggs per treatment per trial.

Embryonic mortality and hatchability

Unhatched eggs were broken out on day 28 and categorized as infertile, early dead, late dead, pipped, or rotten. Results are presented in Table 4 for embryonic mortality and hatchability for all trials. On average for all 3 trials, the sanitized treatment had fewer early dead than the washed treatment ($P = 0.003$) and fewer late dead than both the control ($P < 0.001$) and washed treatments ($P < 0.001$). Overall, the sanitized eggs had the lowest total embryonic mortality (12.96%) when compared to the control (27.82%; $P < 0.001$) and washed (23.35%; $P < 0.001$) treatments. These findings are different than previous research that used the H_2O_2/UV AOP method on chicken hatching eggs which resulted in no embryonic mortality or hatch of fertile differences between the untreated and treated eggs (Wells, et al., 2011b; Fuchs, 2013). This could be due to the highly soiled nature of duck eggs which have higher surface eggshell microbial loads that could penetrate the eggshell through the pores prior to or during incubation.

Table 4. Duck egg embryonic mortality and hatchability for trials 1, 2 and 3

Trial	Treatment	Early dead[1]	Late dead[2]	Pipped	Total embryonic mortality	Rotten	Hatch of fertile
		% ± SE					
1	Control	9.23 ± 1.50[a]	25.45 ± 0.69[a]	9.95 ± 4.34[a]	44.62 ± 4.70[a]	0.51 ± 0.29	54.66 ± 4.51[c]
	Washed	10.07 ± 0.73[a]	14.07 ± 0.94[b]	3.82 ± 0.35[b]	27.95 ± 0.49[b]	1.35 ± 0.34	70.66 ± 0.25[b]
	Sanitized	4.65 ± 1.76[b]	5.54 ± 0.80[c]	3.75 ± 0.53[b]	13.95 ± 3.06[c]	0.84 ± 0.17	85.16 ± 3.11[a]
2	Control	1.79 ± 0.45	7.39 ± 2.00[a]	4.83 ± 0.64	14.02 ± 2.21	1.42 ± 0.10	84.23 ± 2.32
	Washed	4.16 ± 0.66	5.76 ± 1.38[ab]	5.05 ± 1.23	14.97 ± 2.10	0.34 ± 0.17	84.67 ± 1.92
	Sanitized	2.16 ± 0.55	3.23 ± 1.12[b]	3.03 ± 0.44	8.41 ± 1.39	1.52 ± 0.29	89.79 ± 1.44
3	Control	2.81 ± 0.75	12.60 ± 1.81[a]	9.40 ± 2.38	24.82 ± 4.20[ab]	1.35 ± 0.73[ab]	76.22 ± 5.25
	Washed	5.49 ± 1.09	11.77 ± 1.94[a]	9.88 ± 2.31	27.14 ± 3.64[a]	0.51 ± 0.51[b]	73.26 ± 4.29
	Sanitized	4.25 ± 0.79	5.50 ± 0.42[b]	6.75 ± 0.78	16.50 ± 1.54[b]	1.85 ± 0.61[a]	81.20 ± 1.93
Average	Control	4.61 ± 1.13[b]	15.15 ± 2.69[a]	8.06 ± 1.67[a]	27.82 ± 4.64[a]	1.09 ± 0.21	71.70 ± 4.78[b]
	Washed	6.57 ± 0.99[a]	10.53 ± 1.44[b]	6.25 ± 1.20[ab]	23.35 ± 2.43[a]	0.73 ± 0.24	76.19 ± 2.54[b]
	Sanitized	3.69 ± 0.69[b]	4.76 ± 0.56[c]	4.51 ± 0.58[b]	12.96 ± 1.52[b]	1.40 ± 0.25	85.38 ± 1.63[a]

[a-c]Means within a column per trial having different superscripts are significantly different ($P < 0.05$).
[1]Classified as early dead for day 1 to 14 of embryogenesis.
[2]Classified as late dead for day 15 to 28 of embryogenesis.

The average hatch of fertile for the sanitized treatment was greater when compared to both the control and washed ($P < 0.002$) treatments. Previous research has not been published on the effects of H_2O_2/UV AOP method for egg sanitization on duck hatching eggs. However, a study on highly soiled duck hatching eggs that used chlorine foam as a sanitization method demonstrated an increase of 10% hatch of fertile for treated eggs compared to untreated eggs (Patterson, et al., 1990). That experiment reported that the chlorine foam sanitized eggs hatched at an average of 77.8%, which is similar to hatchability obtained with the commercial egg washing method used at the hatchery in this study. The hatchability results in this study indicated that the sanitized treatment resulted in a 13% greater hatch of fertile compared to control, and 9% greater hatch of fertile than the washed treatment. Therefore, the H_2O_2/UV AOP method could be an effective alternative to current commercial egg washing methods used in the commercial duck industry.

Trial 3 moisture loss and duckling weight

Moisture loss for day 1 to 25 of incubation was calculated for Trial 3 only (Table 5). No significant differences were observed between the treatments for egg moisture loss or duckling weight. Moisture loss was 7.67, 8.43, and 9.46% for control, washed and sanitized treatments, respectively. Similar results were observed in a previous study that used the H_2O_2/UV AOP method on chicken hatching eggs which showed no differences in either moisture loss or chick weight (Wells, et al., 2011b). Therefore, it can be concluded that the H_2O_2/UV AOP method does not significantly alter the cuticle of the egg or impact eggshell porosity in any manner that would impact moisture loss from the egg or the hydration status of the resulting hatchlings.

Table 5. Duck egg moisture loss[1] and duckling weight at hatch for Trial 3[2]

Treatment	Moisture loss (%)	Duckling weight (g)
Control	7.67 ± 0.47	61.5 ± 0.02
Washed	8.43 ± 0.09	59.2 ± 0.08
Sanitized	9.46 ± 0.11	60.7 ± 0.09

[1]Moisture loss calculated based on the difference of egg weight on day 0 and 25 of incubation.
[2]n = 3 incubators per treatment.

Duckling quality

Duckling quality assessments for all trials are presented in Table 5. In Trial 1, the sanitized treatment resulted in fewer ducklings with naval tags and leg problems compared to the control, and the washed and sanitized treatments both had fewer cull ducklings compared to the control. In Trial 2, the washed treatment resulted in fewer ducklings with naval tags compared to the control, and in Trial 3 the sanitized treatment had fewer naval tags than the control and washed treatments. On average for all 3 trials, the control had greater naval tags (9.42%) than the sanitized (5.53%; P = 0.002) and washed (6.75%; P = 0.023) treatments. Similarly, when compared to the control, the percent of cull ducklings was lower for the washed (P = 0.024) and sanitized (P = 0.008) treatments. Overall, the percent of good ducklings was higher for the sanitized (91.32%; P = 0.001) and washed (89.72%; P = 0.011) treatments when compared to the control (86.00%). Similar to the embryonic mortality and hatchability results, the greater number of good ducklings for the washed and sanitized treatments might be attributed to the microbial load reductions observed with the washed and sanitized treatments compared to the high microbial load that is naturally present on untreated duck eggs (Table 3).

Table 6. Duckling quality assessment for trials 1, 2 and 3

Trial	Treatment	Naval tags	Leg problems	Dirty feathers	Cull ducklings	Good ducklings[1]
		% ± SE				
1	Control	8.14 ± 1.02[a]	6.84 ± 1.29[ac]	0.00 ± 0.00	3.45 ± 1.21[a]	81.57 ± 2.40[b]
	Washed	4.67 ± 0.28[ab]	5.90 ± 0.73[bc]	0.00 ± 0.00	0.74 ± 0.43[b]	88.69 ± 0.55[a]
	Sanitized	3.20 ± 1.20[b]	3.70 ± 1.63[b]	0.00 ± 0.00	0.42 ± 0.42[b]	92.68 ± 2.47[a]
2	Control	11.52 ± 2.57[a]	0.64 ± 0.37	1.04 ± 0.74	0.49 ± 0.25	86.32 ± 1.97
	Washed	7.43 ± 1.77[b]	0.85 ± 0.55	1.30 ± 0.67	0.21 ± 0.21	90.21 ± 2.02
	Sanitized	9.21 ± 0.93[ab]	2.76 ± 0.76	0.82 ± 0.55	0.00 ± 0.00	87.21 ± 0.97
3	Control	8.59 ± 1.06[a]	0.26 ± 0.26	0.00 ± 0.00	1.02 ± 0.29	90.12 ± 1.35
	Washed	8.27 ± 0.13[a]	0.00 ± 0.00	0.50 ± 0.25	0.98 ± 0.19	90.25 ± 0.26
	Sanitized	4.19 ± 0.85[b]	0.66 ± 0.39	0.22 ± 0.22	0.87 ± 0.44	94.06 ± 0.75
Average	Control	9.42 ± 0.91[a]	2.58 ± 1.15	0.35 ± 0.28	1.65 ± 0.60[a]	86.00 ± 1.64[b]
	Washed	6.79 ± 0.75[b]	2.25 ± 0.96	0.60 ± 0.28	0.64 ± 0.19[b]	89.72 ± 0.66[a]
	Sanitized	5.53 ± 0.88[b]	2.37 ± 0.74	0.35 ± 0.11	0.43 ± 0.22[b]	91.32 ± 1.16[a]

[a-b]Means within a column per trial having different superscripts are significantly different ($P < 0.05$).
[1]Good ducks calculated as a percentage of hatched ducks without naval tags, leg problems, dirty feathers, or culled.

Conclusions

In all three trials, the eggshell surface total aerobes were significantly lower for the sanitized eggs to the control untreated eggs. However, when comparing the H_2O_2/UV AOP method to a common commercial duck egg washing process, microbial loads were similar. However, the sanitized had less total embryonic mortality when compared to both the control and the washed. These results suggest the washing process might not prevent the penetration of microbes into the egg. The sanitized treatment increased hatchability by more than 13% when compared to those eggs left untreated and more than 9% when compared to the washed eggs. These results differ from previously reported research with chicken eggs. These differences in results might be due to the highly soiled nature of the duck eggs, compared to the chicken eggs.

For an egg sanitization to be adopted in the commercial industry, it needs to be economically feasible, able to reduce eggshell contamination without negative impacts on hatchability or bird quality, and must not be hazardous to people or the environment. This study validated that the H_2O_2/UV AOP method, which meets such criteria, is an effective alternative to a common commercial duck hatching egg washing method. In addition, the use of the H_2O_2/UV AOP method to sanitize duck eggs in this study was also found to yield the most consistent results between trials when compared to control and washed treatments.

CHAPTER IV

COMPARISON OF SURFACE AND SUBSURFACE EGGSHELL MICROBIAL LOADS BETWEEN COMMERCIALLY WASHED AND H_2O_2/UV AOP SANITIZED DUCK AND TURKEY HATCHING EGGS

Introduction

Duck and turkey hatching eggs are often highly soiled, and therefore are normally washed in commercial settings to remove adhering organic matter and reduce the microbial loads. It has been suggested that microbial contamination of hatching eggs could lead to an increase in embryonic mortality, decrease in hatchability, poor hatchling quality, and loss in bird performance (Berrang, 1999; Sylte, et al., 2017).

To protect the developing embryo against microbial contamination, the egg consist of antimicrobial components such as the cuticle, inner and outer shell membranes, and the albumen. The cuticle and shell membranes act as a barrier against microbial invasion. It has been noted that due to its tighter meshwork, the inner membrane is more effective at delaying inward microbial invasion than the outer shell membrane (Berrang, 1999). The albumen is viscous, delaying inward movement of bacteria towards the yolk and developing embryo. The albumen also contains antimicrobial proteins such as conalbumin (ovotransferrin), ovomucoid, and lysozyme which decrease survivability of problematic microorganisms (Board and Fuller, 1974; Board and Fuller, 1994; Yamamoto, et al., 1997).

Differences between duck and turkey egg structure and properties might play a role in their susceptibility to microbial contamination (Table 1). Turkey eggs are generally larger in size and weight when compared to duck eggs (Brown, et al., 1965). Relative density of the shell membranes is also different between duck and turkey eggs with duck eggs having higher shell

membrane relative density than turkey eggs. This implies the shell membranes of duck eggs are more dense than those of turkey eggs (Brown, et al., 1965). However, the thickness of the inner and outer shell membranes of turkey eggs are greater than those of duck eggs (Brown, et al., 1965). In addition, the Haugh unit of turkey eggs is higher when compared to duck eggs (Juárez-Caratachea, et al., 2011; OnbaŞılar, et al., 2011; Popoola, et al., 2015). Overall, this information suggests that turkey and duck eggs might differ in their susceptibility to microbial invasion due to differences in egg structure.

Large amounts of poultry production at a high rate could increase the incidence of pathogenic contamination of poultry products (Board and Fuller, 1974). Besides *Salmonella,* potential pathogens of poultry include, *Escherichia coli, Pseudomonas, Enterococcus, Staphylococcus,* and fungal microorganisms. Some authors have suggested that *Escherichia coli* is the primary pathogen of turkeys and is associated with several secondary infectious diseases in poultry (Montgomery, et al., 1999). *Pseudomonas* spp. are associated with secondary infectious diseases in poultry. Egg contamination by those bacteria can lead to a high percent of rotten eggs in hatcheries (Board and Fuller, 1974; Dinev, et al., 2013; Badr, et al., 2016). *Enterococcus* spp. may cause acute septicemia and cellulitis in poultry (Morishita, 2018). *Staphylococcus* is naturally found in poultry species. *Staphylococcus* infection may cause a broad spectrum of diseases such as impetigo, abscesses, or more fatal diseases such as toxic shock syndrome. *Staphylococcus aureus* is also known to be zoonotic and cause foodborne illness in humans (Shareef, et al., 2009; Li, et al., 2016).

Fungi are also problematic microorganisms that naturally grow under hatching cabinet conditions (high humidity and warm temperature). Fungi species are able to bypass the cuticle, shell membranes, and viscous nature of the albumen. The hyphae of fungi are able to penetrate

through the eggshell pores and through the shell membranes of the egg, while the mycelia of micro-fungi are able to grow and ramify through the viscous albumen. If contamination by fungi occurs, it may cause high embryonic mortality (Board and Fuller, 1974; Board and Fuller, 1994).

Previous studies have indicated that the use of the H_2O_2/UV advanced oxidation process (AOP) method of egg sanitization has demonstrated to be highly effective for reduction of surface microbial loads (Wells, et al., 2010; Wells, et al., 2011b; Al-Ajeeli, et al., 2016; Rehkopf, et al., 2017). An AOP is an aqueous phase process that causes oxidation and leads to inactivation of pathogenic cells through the action of hydroxyl radicals (Legrini, et al., 1993; Comninellis, et al., 2008). Photolysis of the peroxide bond in H_2O_2 yields hydroxyl radicals that have an unpaired electron that easily interacts with vital cellular components such as lipids, proteins, DNA, and carbohydrates to ultimately cause cell death (Shimoda, et al., 1997; Mamane, et al., 2007; Ikai, et al., 2010).

The objectives of the experiments described in this chapter were: 1) to compare the effectiveness of the H_2O_2/UV AOP treatment to commercial egg washing practices on the surface and subsurface eggshell microbial loads on duck and turkey hatching eggs, 2) further evaluate the H_2O_2/UV AOP method of egg sanitization by treating duck hatching eggs with one and two passes through a prototype egg sanitizer applying the H_2O_2/UV AOP treatment and, 3) investigate the effects of the H_2O_2/UV AOP treatment of egg sanitization alone or in combination with the standard egg washing practices in a commercial hatchery setting.

Materials and methods

Treatments

Experiment 1. Two trials were conducted in this experiment using commercial white Pekin duck (Trial 1) and turkey (Trial 2) hatching eggs that were obtained from a commercial

duck and turkey breeder company, respectively. Treatments evaluated were control, washed, and sanitized. The control treatment consisted of eggs that did not undergo any form of washing or sanitization. The washed treatment consisted of eggs that were washed at a commercial duck hatchery or turkey breeder farm using an egg washing machine filled with a combination of water, detergents, disinfecting agents, and an anti-foaming ingredient. The exact wash water composition was not provided by the turkey and duck companies. The sanitized treatment consisted of unwashed eggs (same as the control treatment) that were randomly selected and treated with the H_2O_2/UV AOP prototype egg sanitizer at Texas A&M University. Hydrogen peroxide and reverse osmosis (RO) water were pre-heated in an incubator set at 37°C and combined to yield 3% H_2O_2 concentration prior to egg treatment. The H_2O_2/UV AOP egg sanitization mechanism consisted of two repetitions of the combination of 3% H_2O_2 spray followed by immediate UV exposure as described in Al-Ajeeli, et al. (2016). Upon exiting the sanitizer, the eggs were allowed to sit for approximately 30 sec prior to conveying them through the sanitizer a second time to assist in removal of adhering organic material. Thus, sanitized eggs were exposed to 4 total combinations of H_2O_2 and UV treatment. Upon final exit of the prototype sanitizer, sanitized treated eggs were allowed to sit for approximately 3 min prior to sampling.

Experiment 2. Two trials were conducted in this experiment using commercial white Pekin duck eggs that were obtained from the same commercial duck breeder company as in Experiment 1 Trial 1 evaluated control, washed, sanitized once, and sanitized twice treatments. The control treatment consisted of eggs that did not undergo any form of washing or sanitization. The washed treatment consisted of eggs that were washed at a commercial duck hatchery as in Experiment 1. Sanitized once and sanitized twice treatments consisted of unwashed eggs that were randomly selected for treatment with the H_2O_2/UV AOP prototype egg sanitizer at Texas

A&M University. The sanitized once treated eggs were conveyed through the prototype sanitizer once. Thus, sanitized once treated eggs were exposed to 2 total combinations of 3% H_2O_2 followed by UV treatment. The sanitized twice treated eggs were conveyed through the sanitizer twice. Thus, sanitized twice eggs were exposed to 4 total combinations of 3% H_2O_2 followed by UV treatment. Upon final exit from the prototype sanitizer, sanitized once and twice treated eggs were allowed to sit for approximately 3 min prior to sampling. Trial 2 evaluated a control, washed, and washed + sanitized treatment. As previously mentioned, the control treatment consisted of eggs that did not undergo any form of washing or sanitization. The washed treatment consisted of eggs that were washed at the commercial duck hatchery. The washed + sanitized treatment was also performed at the commercial duck breeder facility. First, eggs were washed using the commercial duck breeder's washing process, and were allowed to air dry prior to conveying them once through the prototype H_2O_2/UV egg sanitizer machine that had been transported to the hatchery. Therefore, eggs were washed and then exposed to 2 combinations of H_2O_2/UV treatment. Table 7 summarizes the species, treatments, and media evaluated per experiment and trial.

Table 7. Species, treatments, site of treatment, and media evaluated per trial of experiment

Experiment	Trial	Species	Treatments	Site of treatment	Media
1	1	Duck	Control	None	APC Petrifilm
			Washed	Hatchery[1]	Yeast & Mold Petrifilm
			Sanitized	TAMU[2]	*E. coli* / Total coliform Petrifilm
					MacConkey Agar
					Sabourad Dextrose Agar
					Bile Esculin Azide Agar
					Mannitol Salt Agar
	2	Turkey	Control	None	Same as Trial 1
			Washed	Breeder farm[3]	
			Sanitized	TAMU[2]	
2	1	Duck	Control	None	APC Petrifilm
			Washed	Hatchery[1]	Yeast & Mold Petrifilm
			Once sanitized	TAMU[2]	
			Twice sanitized	TAMU[2]	
	2	Duck	Washed	Hatchery[1]	APC Petrifilm
			Sanitized	Hatchery[1]	*Staphylococcus* Petrifilm
			Washed + sanitized	Hatchery[1]	

[1] Eggs were transported from a commercial duck breeder farm to a commercial duck hatchery where the eggs were washed.
[2] Eggs treated at Texas A&M University in laboratory.
[3] Eggs washed immediately after collection at a commercial turkey breeder farm.

Microbial Analysis

Surface microbial enumeration. Ten eggs were randomly selected per treatment per trial to evaluate surface and subsurface eggshell microbial loads. All eggs were sampled at Texas A&M University using tongs that were sterilized by dipping them in 100% ethanol followed by flaming with the exception of Trial 2 in Experiment 2 where eggs were sampled at the commercial duck breeder facility, then shipped to Texas A&M University in individual Whirl-Pak bags (Nasco, Fort Atkinson, WI) for microbial analysis. Sampled eggs were placed in Whirl-Pak bags containing 25 mL of sterile PBS (pH 7.4; HiMedia Laboratories, West Chester, PA). Eggs were massaged by hand for 1 min in the bag. Ten-fold serial dilutions were performed. For Petrifilms, a volume of 1 mL of each egg rinse solution and dilution was plated, and for agars a volume of 0.1 mL was spread onto media using sterile plastic spreaders (VWR International LLC, Radnor, PA). After inoculation, media were incubated. Colonies were then enumerated and calculated as $\log_{10}$cfu/egg. Therefore, the limit of detection (LOD) for Petrifilms was 25 cfu/egg, or 1.398 $\log_{10}$cfu/egg, and for agars it was 250 cfu/egg, or 2.398 $\log_{10}$cfu/egg. Volume plated, LOD, and incubation temperature (°C) and time (h) are shown on Table 8 per media evaluated.

Subsurface microbial enumeration. The same eggs that were sampled for surface microbial load enumeration were used to evaluate subsurface microbial loads. Under a biosafety cabinet, a modified crush and rub technique was performed (Figure 2). Eggs were removed from the Whirl-Pak bag, rinsed with sterile PBS, and scrubbed with a sterile sponge saturated with PBS to remove adhering microorganisms from the eggshell surface. After rinsing with PBS a second time, eggs were then placed in a new Whirl-Pak bag containing 25 mL of PBS. After massaging eggs vigorously for about 1 min, rinsate was plated onto APC Petrifilms to ensure microorganisms were removed from the surface of the shell. Next, eggs were rinsed with PBS a

third time, cracked, and contents removed. Sterile PBS was used to remove any adhering albumen from the inside of the shell. The eggshell was then placed in a sterile centrifuge tube containing 25 mL of PBS and vigorously crushed with a sterilized spatula. Ten-fold serial dilutions were performed and 1 mL (Petrifilms) or 0.1 mL (agars) was plated onto the previously mentioned media, incubated (Table 8), and enumerated. Colonies were calculated as log_{10}cfu/egg. Therefore, the limit of detection for this assay was 25 cfu/egg, or 1.398 log_{10}cfu/egg for Petrifilms, and 250 cfu/egg or 2.398 log_{10}cfu/egg for agars.

Figure 2. Modified crush and rub technique performed for subsurface microbial load enumeration

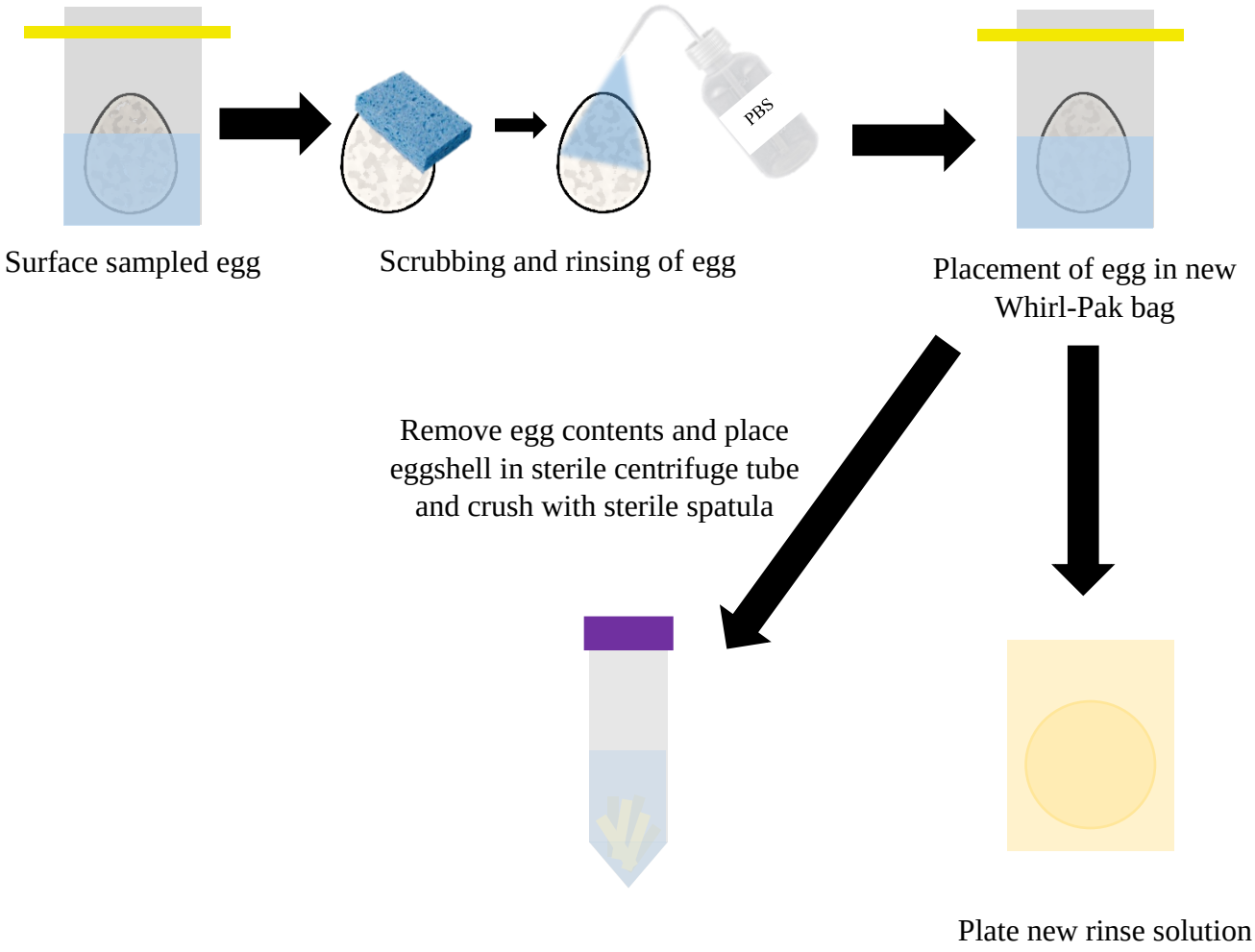


Table 8. All media and corresponding microorganisms evaluated, volume plated, limit of detection (LOD), and incubation time and temperature in Experiment 1

Media	Supplier	Organism	Volume plated	LOD[1]	Incubation time and temperature[2]
APC Petrifilm	3M United States, Maplewood, MN	Total Aerobes	1 mL	25	48 h at 37°C
Yeast & Mold Petrifilm	3M United States, Maplewood, MN	Yeast and Mold	1 mL	25	5 d at 25°C
E. coli / coliform count Petrifilm	3M United States, Maplewood, MN	*Escherichia coli* / total coliforms	1 mL	25	24 to 48 h at 37°C
Staphylococcus Petrifilm	3M United States, Maplewood, MN	*Staphylococcus* spp.	1 mL	25	24 h at 37°C
MacConkey Agar	BD Difco BBL Microbiology Distributor, Houston, TX	Gram-negative enteric organisms	0.1 mL	250	48 h at 37°C
Sabourad Dextrose Agar	BD Difco BBL Microbiology Distributor, Houston, TX	Fungi and Aciduric microorganism	0.1 mL	250	5 d at 25°C
Bile Esculin Agar	Criterion, Hardy Diagnostics, Santa Maria, CA	*Enterococcus*	0.1 mL	250	48 h at 37°C
Mannitol Salt Agar	BD Difco BBL Microbiology Distributor, Houston, TX	*Staphylococci*	0.1 mL	250	48 h at 37°C

[1]LOD indicates the lowest concentration of microorganisms that could be calculated given the initial volume plated per media for accurate cfu/egg enumeration.
[2]As directed by media manufacturer's instructions.

Statistical analysis

Eggs that yielded zero colony counts were assigned a value of 1.097 $\log_{10}$cfu/egg for Petrifilms and 2.097 $\log_{10}$cfu/egg for agars for statistical analysis. Data collected was analyzed as a one-way ANOVA using a model of y=treatment. If significance was detected, means were separated by an LSD post hoc test, with significance of $P < 0.05$.

Results and discussion

Microbial enumeration

Experiment 1, Trial 1. Results for Trial 1 of Experiment 1 are presented in Table 9. Results for surface APC for duck hatching eggs demonstrated that the sanitized (3.93 $\log_{10}$cfu/egg) and washed (3.45 $\log_{10}$cfu/egg) eggs had reduced APC compared to the control (5.63 $\log_{10}$cfu/egg; $P < 0.02$) eggs. The subsurface APC for the sanitized (3.09 $\log_{10}$cfu/egg) treatment were reduced when compared to both the control (4.34 $\log_{10}$cfu/egg; $P = 0.016$) and washed (4.31 $\log_{10}$cfu/egg; $P = 0.02$) treatments. These results indicate that the use of a common commercial egg washing method is effective at reducing surface eggshell microbial loads. However, it does not significantly reduce microbes inside the pores and shell membranes of the egg (subsurface). In contrast, the H_2O_2/UV AOP method was effective at reducing both surface and subsurface microbial loads. Furthermore, results from this trial demonstrated that surface total coliforms, fungi and aciduric microorganisms, *Enterococcus*, and *Staphylococcus aureus*, were lower for the sanitized treatment compared to the control treatment ($P < 0.003$). There were no additional differences detected between treatments for subsurface microbial loads for any media evaluated.

Experiment 1, Trial 2. Results for Trial 2 of Experiment 1 are presented on Table 10. Similar to Trial 1, surface APC results for turkey hatching eggs demonstrated that the sanitized

(2.89 $\log_{10}$cfu/egg) treatment was reduced when compared to the washed (4.47 $\log_{10}$cfu/egg; $P < 0.001$) and control (5.67 $\log_{10}$cfu/egg; $P < 0.001$) treatments. For subsurface APC, the sanitized (1.50 $\log_{10}$cfu/egg) treatment was lower than both the washed (2.41 $\log_{10}$cfu/egg; $P = 0.038$) and control (2.82 $\log_{10}$cfu/egg; $P = 0.004$) treatments. Similar to Trial 1 results, using a common commercial turkey egg washing process was effective at reducing surface microbial loads. However, that method did not reduce subsurface microbial loads. Also similar to Trial 1 results, the H_2O_2/UV AOP method on turkey hatching eggs was reduced for both surface and subsurface microbial loads. Additionally, results indicated that the sanitized and washed treatments had reduced surface microbial loads compared to the control for *Escherichia coli* ($P < 0.001$), total coliforms ($P < 0.001$), yeast and mold on Petrfilms ($P < 0.001$), fungi and aciduric microorganisms ($P < 0.001$), and *Staphylococcus enteritidis* ($P < 0.001$). Results also demonstrated that the sanitized treatment was reduced when compared to both the washed ($P < 0.001$) and control ($P < 0.040$) treatments for E*nterococcus*, and S*taphylococcus aureus*. No additional differences were observed between treatments for subsurface microbial loads on any evaluated media.

Overall, data from Experiment 1 indicated that the use of the H_2O_2/UV AOP method of egg sanitization is effective at reducing both surface and subsurface APC for both duck and turkey hatching eggs. Therefore, differences in duck and turkey egg structure could have no influence on the efficiency of hydroxyl radical interaction with microorganisms in the pores and shell membranes of the egg. However, there were noted differences between surface microbial reductions between duck and turkey eggs. This might be due to differences in cuticle structure, egg components, or organic matter surrounding the eggshell. However, few studies have been

conducted to evaluate the differences between cuticle structure and components between poultry species.

Table 9. Duck eggshell surface and subsurface microbial loads (log_{10} cfu/egg ± SE) for Experiment 1, Trial 1[1]

Media	Organism	Treatment	Surface	Subsurface
Petrifilm	Total aerobes (APC)	Control	5.63 ± 0.12[a]	4.34 ± 0.33[a]
		Washed	3.45 ± 0.31[b]	4.31 ± 0.45[a]
		Sanitized	3.93 ± 0.26[b]	3.09 ± 0.19[b]
Petrifilm	*Escherichia coli*	Control	2.05 ± 0.41	2.40 ± 0.54
		Washed	1.70 ± 0.34	2.58 ± 0.51
		Sanitized	1.10 ± 0.00	1.52 ± 0.19
Petrifilm	Total coliforms	Control	2.92 ± 0.49[a]	2.62 ± 0.62
		Washed	1.97 ± 0.45[ab]	3.10 ± 0.54
		Sanitized	1.10 ± 0.00[b]	1.99 ± 0.28
Petrifilm	Yeast and mold	Control	2.53 ± 0.37	2.43 ± 0.50
		Washed	1.65 ± 0.34	2.39 ± 0.46
		Sanitized	1.19 ± 0.05	1.64 ± 0.20
Sabouraud Dextrose Agar	Fungi and aciduric organisms	Control	4.42 ± 0.26[a]	3.56 ± 0.42
		Washed	2.85 ± 0.33[b]	3.72 ± 0.44
		Sanitized	2.16 ± 0.04[b]	2.75 ± 0.17
Bile Esculin Agar	*Enterococcus*	Control	4.61 ± 0.22[a]	2.64 ± 0.28
		Washed	2.75 ± 0.33[b]	2.61 ± 0.25
		Sanitized	2.65 ± 0.19[b]	2.10 ± 0.00
Mannitol Salt Agar	*Staphylococcus* spp.	Control	4.86 ± 0.11[a]	2.27 ± 0.31[ab]
		Washed	2.31 ± 0.25[c]	2.61 ± 0.36[a]
		Sanitized	2.88 ± 0.24[b]	2.10 ± 0.00[b]
MacConkey Agar	Gram-negative organisms	Control	2.91 ± 0.28	3.26 ± 0.48
		Washed	2.63 ± 0.28	3.70 ± 0.41
		Sanitized	2.10 ± 0.00	2.50 ± 0.18

[a-c] Means within a column per media having different superscripts are significantly different ($P < 0.05$).

[1] n = 10 eggs per treatment.

Table 10. Turkey eggshell surface and subsurface microbial loads (log_{10} cfu/egg ± SE) for Experiment 1, Trial 2[1]

Media	Organism	Treatment	Surface	Subsurface
Petrifilm	Total aerobes	Control	5.67 ± 0.09[a]	2.82 ± 0.26[a]
		Washed	4.47 ± 0.14[b]	2.41 ± 0.39[a]
		Sanitized	2.89 ± 0.45[c]	1.50 ± 0.21[b]
Petrifilm	*Escherichia coli*	Control	1.45 ± 0.10[a]	1.10 ± 0.00
		Washed	1.10 ± 0.00[b]	1.52 ± 0.43
		Sanitized	1.10 ± 0.00[b]	1.10 ± 0.00
Petrifilm	Total coliforms	Control	2.81 ± 0.21[a]	2.29 ± 0.09
		Washed	1.46 ± 0.17[b]	1.84 ± 0.41
		Sanitized	1.10 ± 0.00[b]	1.13 ± 0.03
Petrifilm	Yeast and mold	Control	4.28 ± 0.24[a]	1.54 ± 0.16
		Washed	2.29 ± 0.20[b]	1.40 ± 0.12
		Sanitized	1.16 ± 0.04[b]	1.22 ± 0.08
Sabouraud Dextrose Agar	Fungi and aciduric organisms	Control	4.21 ± 0.20[a]	2.54 ± 0.12
		Washed	2.63 ± 0.11[b]	2.47 ± 0.30
		Sanitized	2.28 ± 0.08[b]	2.10 ± 0.00
Bile Esculin Agar	*Enterococcus*	Control	5.58 ± 0.15[a]	2.71 ± 0.22
		Washed	4.05 ± 0.21[b]	2.57 ± 0.24
		Sanitized	2.84 ± 0.34[c]	2.13 ± 0.03
Mannitol Salt Agar	*Staphylococcus* spp.	Control	5.68 ± 0.25[a]	2.80 ± 0.24
		Washed	4.16 ± 0.19[b]	2.48 ± 0.24
		Sanitized	3.02 ± 0.32[c]	2.20 ± 0.11
MacConkey Agar	Gram-negative organisms	Control	2.75 ± 0.38	2.10 ± 0.00
		Washed	2.30 ± 0.21	2.41 ± 0.32
		Sanitized	2.10 ± 0.00	2.10 ± 0.00

[a-c] Means within a column per media having different superscripts are significantly different ($P < 0.05$).

[1] n = 10 eggs per treatment.

Experiment 2 Trial 1. Several eggs in this trial yielded subsurface counts that were greater than expected; thus, some Petrifilms were not able to be counted (too numerous to count). Therefore, statistical analysis was not able to be performed. Raw data presented as $\log_{10}$cfu/egg is shown in Table 11a for APC and Table 11b for yeast and mold counts. In general, the surface APC data demonstrated that the washed and sanitized treatments had lower counts than the control treatment. For subsurface APC, the washed treatment appeared to have the greatest microbial loads when compared to the other treatments because 4 eggs were too numerous to count (TNTC). These results further imply that a common commercial egg washing process is effective at reducing surface microbial loads but does not reduce subsurface microbes, and in this case, may increase the subsurface counts compared to the control. Results for surface and subsurface yeast and mold counts (Table 11b) indicate that the twice sanitized treatment likely had lower counts compared to the control, washed, and once sanitized treatments. Results from this trial are similar to Experiment 1.

Experiment 2, Trial 2. The data for this trial are presented in Table 12. Results only indicated differences between treatments for surface microbial loads. No differences between treatments were observed for subsurface on any of the media evaluated. The washed + sanitized (1.42 $\log_{10}$cfu/egg) treatment had the lowest APC compared to the washed (3.13 $\log_{10}$cfu/egg; $P = 0.024$) and sanitized (4.65 $\log_{10}$cfu/egg; $P < 0.001$) treatments. Surface *Staphylococcus* spp. results demonstrated that the washed + sanitized (1.09 $\log_{10}$cfu/egg) treatment was also lower compared to the washed (2.47 $\log_{10}$cfu/egg; $P = 0.040$) and sanitized (3.64 $\log_{10}$cfu/egg; $P < 0.001$) treatments. However, yeast and mold results indicated that the washed (1.13 $\log_{10}$cfu/egg) and washed + sanitized (1.16 $\log_{10}$cfu/egg) treatments had lower microbial counts compared to the sanitized (1.58 $\log_{10}$cfu/egg; $P < 0.016$) treatment. The washed + sanitized treatment

demonstrated that in general, washing duck hatching eggs with a standard commercial egg washing process in combination with the H_2O_2/UV AOP sanitization method was effective at reducing surface microbial loads. The washed + sanitized method could be the best commercially feasible egg sanitization method for highly soiled eggs because the washing process could initially remove adhering organic material and the H_2O_2/UV AOP method could then inactivate microorganisms that were not previously removed from the surface and subsurface of the eggshell. Further research should be conducted to evaluate the effects of the washed + sanitized method of egg sanitization on hatchability and overall hatchling quality.

Table 11a. Raw data for duck eggshell surface and subsurface (log_{10} cfu/egg) APC for Experiment 2, Trial 1.

Treatment egg number	Egg number	Surface	Subsurface
Control	1	6.61	3.70
	2	6.54	4.34
	3	6.33	4.40
	4	6.93	4.23
	5	5.85	3.40
	6	6.22	4.24
	7	6.23	TNTC
	8	6.52	4.07
	9	6.44	4.75
	10	5.26	3.40
	Average	6.29	NC[2]
Washed	1	4.97	TNTC
	2	5.20	TNTC
	3	5.12	TNTC
	4	1.10	3.40
	5	1.10	2.94
	6	1.10	2.68
	7	1.10	2.98
	8	1.10	4.60
	9	1.10	N/A[1]
	10	4.90	TNTC
	Average	2.68	NC[2]
Sanitized once	1	3.38	TNTC
	2	3.83	4.39
	3	3.15	2.95
	4	5.51	4.59
	5	5.51	1.10
	6	3.63	3.74
	7	4.37	2.60
	8	5.30	TNTC
	9	4.98	4.26
	10	3.86	3.90
	Average	4.35	NC[2]
Sanitized twice	1	2.10	4.13
	2	4.05	3.18
	3	4.24	4.17
	4	2.81	2.51
	5	1.70	2.30
	6	4.45	4.36
	7	5.50	TNTC
	8	4.74	4.39
	9	4.90	4.65
	10	4.01	4.33
	Average	3.85	NC[2]

[1]Washed egg number 9 was cracked upon arrival and therefore subsurface APC were not obtained.
[2]Average subsurface eggshell APC were not able to be calculated due to lack of colony counts on individual eggs.

Table 11b. Raw data for duck eggshell surface and subsurface (log_{10} cfu/egg) yeast and mold counts for Experiment 2, Trial 1

Treatment	Egg Number	Surface	Subsurface
Control	1	2.24	2.85
	2	2.44	2.98
	3	1.70	3.15
	4	2.18	2.10
	5	1.40	2.44
	6	2.18	2.51
	7	2.18	2.00
	8	2.51	1.88
	9	2.00	2.51
	10	1.40	2.24
	Average	2.02	2.47
Washed	1	3.49	5.03
	2	3.20	4.11
	3	1.10	2.60
	4	1.10	1.88
	5	1.10	2.00
	6	1.10	1.88
	7	1.10	2.57
	8	1.10	1.10
	9	1.10	N/A[1]
	10	1.10	1.10
	Average	1.55	2.47[2]
Sanitized once	1	1.40	1.70
	2	1.10	1.40
	3	1.10	1.10
	4	1.70	3.68
	5	1.10	1.40
	6	1.40	1.40
	7	1.40	1.10
	8	2.30	TNTC
	9	1.70	1.40
	10	1.10	2.10
	Average	1.43	NC[3]
Sanitized twice	1	1.10	1.10
	2	1.10	1.40
	3	1.10	2.51
	4	1.10	1.10
	5	1.10	1.10
	6	1.70	1.10
	7	1.88	1.70
	8	1.40	1.70
	9	1.10	3.50
	10	1.10	1.10
	Average	1.27	1.63

[1]Washed egg number 9 was cracked upon arrival and therefore subsurface yeast and mold counts were not obtained.
[2] Average was calculated based on the 9 eggs that were enumerated for subsurface yeast and mold counts.
[3]Average subsurface eggshell yeast and mold counts were not able to be calculated due to lack of colony counts on individual eggs.

Table 12. Duck egg surface and subsurface (log_{10} cfu/egg ± SE) microbial counts for Experiment 2, Trial 2[1]

Media	Organism	Treatment	Surface	Subsurface
Petrifilm	Total Aerobes (APC)	Washed	3.13 ± 0.46[b]	2.76 ± 0.48
		Sanitized	4.65 ± 0.57[a]	3.45 ± 0.46
		Washed + Sanitized	1.42 ± 0.26[c]	2.38 ± 0.51
Petrifilm	*Staphylococcus* spp.	Washed	2.47 ± 0.45[a]	1.72 ± 0.31
		Sanitized	3.64 ± 0.64[a]	1.51 ± 0.28
		Washed + Sanitized	1.09 ± 0.00[b]	1.36 ± 0.26
Petrifilm	Yeast and Mold	Washed	1.13 ± 0.03[b]	1.25 ± 0.07
		Sanitized	1.58 ± 0.19[a]	1.16 ± 0.04
		Washed + Sanitized	1.16 ± 0.04[b]	1.19 ± 0.05

[a-c] Means within a column per media having different superscripts are significantly different ($P < 0.05$).
[1] n = 10 eggs per treatment per media evaluated.

Conclusions

Sanitization of hatching eggs is an important intervention step for preventing microbial contamination of the developing embryo. Currently, the commercial duck and turkey breeder industries typically wash hatching eggs to decrease surface microbial loads which increases hatchability and improves hatchling quality. This study indicated that a common duck and turkey commercial egg washing method was effective at reducing surface microbial loads; however, no reduction of subsurface microbial loads were observed when compared to untreated control eggs. This finding could be due to the lack of interaction between the detergents and sanitizers in the washing systems with microorganisms inside the pores and shell membranes. It can be concluded from Experiment 1 results that the use of the H_2O_2/UV AOP method was effective at eggshell microbial load reduction when treating eggs in a controlled environment in the laboratory. However, applying the H_2O_2/UV AOP method in a commercial setting proved to be less efficient as was observed in the difference in microbial load results obtained in Experiment 2. In addition, Experiment 2 results also concluded that the combination of a commercial egg washing process followed by 1 pass through the prototype egg sanitizer yielded greater reductions for surface microbial loads compared to 2 passes through the egg sanitizer at the commercial hatchery. Additional research should be conducted to further evaluate the effects of the washed + sanitized treatment (combination of a commercial egg washing process followed by the H_2O_2/UV AOP method) on hatchability and hatchling quality.

Overall, this study also implied that differences between duck and turkey eggshell structure and components might play a role in potential microbial contamination. Limited research has been published in regard to cuticle differences between poultry species. Such information would aid in the interpretation of this study's results and how surface

microorganisms and hydroxyl radicals interact with cuticle components. That information would also be valuable to further design an egg sanitization process that best suits individual poultry species in a commercially feasible manner.

CHAPTER V

EFFECTIVENESS OF TURKEY HATCHING EGG SANITIZATION WITH THE H_2O_2/UV ADVANCED OXIDATIVE PROCESS

Introduction

Turkey eggs are highly soiled in nature and contain high amounts of surface eggshell microbial loads. Microorganisms on the surface of the eggshell can penetrate the eggshell via contraction of the egg contents that occurs during egg cooling after the egg is laid or when placed in a storage cooler (Berrang, 1999; Wells, et al., 2010). Microbial contamination of hatching eggs is not uncommon and can affect hatchability, hatchling quality, and follow a flock to the grow-out farm and cause disease (Berrang, 1999). Therefore, turkey eggs are typically washed in commercial breeder operations. Quaternary ammonium compounds (QAC) are commonly used as disinfecting agents in commercial turkey egg washing processes.

The effects of QAC as a method of egg sanitization on turkey hatching eggs was evaluated in a study conducted by Arhienbuwa, et al. (1980). That study demonstrated a significant reduction of microbial loads on the eggshell surface compared to eggs that were not treated or fumigated with formalin. However, a study conducted by Al-Ajeeli, et al. (2016) compared QAC to the H_2O_2/UV AOP method on chicken eggs and determined that the H_2O_2/UV AOP method was more effective at reducing microbial loads on the surface of the eggshell than QAC. Additional studies have indicated that using the H_2O_2/UV AOP method of egg sanitization is highly effective for reducing eggshell microbial loads (Wells, et al., 2010; Wells, et al., 2011b; Al-Ajeeli, et al., 2016; Rehkopf, et al., 2017). The combined application of H_2O_2 and UV forms an advanced oxidation process (AOP) reaction that is highly antimicrobial. An AOP is an aqueous phase process that causes oxidation and leads to inactivation of pathogenic cells through

the action of hydroxyl radicals (Legrini, et al., 1993; Comninellis, et al., 2008). Photolysis of the peroxide bond in H_2O_2 yields hydroxyl radicals that have an unpaired electron that easily interacts with vital cellular components such as lipids, proteins, DNA, and carbohydrates to ultimately cause cell death (Shimoda, et al., 1997; Mamane, et al., 2007; Ikai, et al., 2010).

Previous studies have not been conducted evaluating the effects of the H_2O_2/UV AOP as a turkey hatching egg sanitization method on eggshell microbial loads, hatchability, and poult quality. The objective of this study was to compare the use of the H_2O_2/UV AOP as a turkey hatching egg sanitization method to a conventional commercial egg washing method and evaluate eggshell microbial loads, embryonic mortality, hatchability and poult quality.

Materials and methods

Treatments

One trial was conducted using turkey hatching eggs obtained from a commercial breeder flock that was 52 weeks of age. Three treatments were evaluated. The control treatment consisted of untreated eggs that did not undergo any form of washing or sanitization. The washed treatment consisted of eggs that were washed at a commercial turkey breeder farm. The sanitized treatment consisted of unwashed control eggs that were treated with the H_2O_2/UV AOP prototype egg sanitizer at Texas A&M University. Hydrogen peroxide and reverse osmosis (RO) water were pre-heated in an incubator set at 37°C and combined to yield 3% H2O2 concentration prior to egg treatment. The H_2O_2/UV AOP egg sanitization mechanism consisted of two repetitions of the combination of 3% H_2O_2 spray followed by immediate UV light exposure. The mechanism consisted of two repetitions of the combination of 3% H_2O_2 followed by immediate UV exposure as described in Al-Ajeeli, et al. (2016). Upon exiting the sanitizer, the eggs were allowed to sit for approximately 30 sec prior to conveying them through the sanitizer a second

time to assist in removal of adhering organic material. Thus, sanitized eggs were exposed to 4 total applications of H_2O_2 and UV.

Microbial analysis

Ten eggs were randomly selected per treatment to evaluate eggshell microbial loads. Eggs were sampled using tongs that were sterilized by dipping them in 100% ethanol followed by flaming. Sampled eggs were placed in Whirl-Pak bags (Nasco, Fort Atkinson, WI) with 25 mL of sterile PBS (pH 7.4; HiMedia Laboratories, West Chester, PA). Eggs were massaged by hand for 1 min in the bag. Ten-fold serial dilutions were performed, and 1 mL of each egg rinse solution and dilution was plated onto aerobic plate count (APC) Petrifilms (3M United States, Maplewood, MN). After 48 h of incubation at 37°C, colonies were enumerated and total APC were calculated as $\log_{10}$cfu/egg. Therefore, the limit of detection for this assay was 25 cfu/egg, or 1.398 $\log_{10}$cfu/egg. A value of 1.097 $\log_{10}$cfu/egg was assigned to eggs with zero counts for statistical analysis.

Incubation and hatching

Three incubators (Model 1500; GQF Manufacturing Company Inc., Savannah, GA) with 4 paired hatchers (Model 1550; GQF Manufacturing Company Inc., Savannah, GA) were utilized for each treatment. However, due to a mechanical failure of 1 incubator in the control group, data were obtained from 2 incubators with 3 paired hatchers. One additional hatcher per treatment was used to accommodate modifications to the hatching trays to meet turkey poult hatching standards as suggested by a commercial turkey breeder. Hatcher trays were modified to allow for additional height between trays for poults to stand, and fewer eggs were placed in each tray than was previously done with duck eggs. Thus, an additional hatcher was needed per treatment to accommodate all the eggs from 3 incubators. Approximately 198 eggs were placed

per incubator, with some variation depending on the number of eggs damaged during transport and handling. The assignment of incubator and paired hatcher and the corresponding number of eggs set per treatment are presented in Table 13.

Table 13. Turkey eggs placed in incubators and hatchers per treatment

Treatment	Incubator number	Hatcher number	Number of eggs[1]	Total number of eggs set
Control	2, 7	2, 4, 7	198,198	396
Washed	1, 5, 10	1, 5, 10, 11	198,198,194	590
Sanitized	3, 8, 12	3, 6, 8, 12	198,198,198	594

[1]Corresponds with incubator number, respectively.

Following treatment application, turkey eggs were placed in incubators at a temperature of 37.7°C and relative humidity of 55%. Temperature and relative humidity during the incubation period were then decreased every 5 d for 25 d as shown in Table 14. On day 26, during egg transfer to hatchers, eggs were candled to remove obvious infertile and non-developing eggs. These eggs were broken out, classified, and data are recorded. Eggs with developing embryos were then placed in hatchers at a temperature of 37.3°C and relative humidity of 55 to 60% (Table 14). On day 28, hatched poults were enumerated and recorded per incubator. Poults were weighed in trays to assess average hatchling weight. The remaining unhatched eggs were broken out and classified as infertile, early dead (0 d to 14 d), late dead (15 d to 28 d), pipped, or rotten. Lastly, all hatched ducklings were examined for quality issues such as naval tags, dirty feathers, or cull poults due to other visible deformities.

Table 14. Turkey incubator and hatching temperature and relative humidity conditions

Day	Temperature	Humidity
0 – 5	37.7°C	55%
6 – 12	37.6°C	50%
13 – 19	37.5°C	50%
20 – 25	37.4°C	50%
26 – 28	37.3°C	55 – 60%

Egg moisture loss

Percent moisture loss of eggs during incubation was also evaluated in this experiment. Prior to incubation, empty egg flats were numbered and weighed. Turkey eggs were placed on these egg flats and weighed. Prior to candling eggs for hatcher placement on day 26, each flat of turkey eggs was weighed again. Egg moisture loss for the first 25 d of incubation was calculated as a percentage of the beginning egg weight for each flat of eggs.

Statistical analysis

Data were analyzed as a one-way ANOVA using the model y=treatment. Assumptions of equal variance and normality were met for ANOVA with means separated by LSD post hoc test. Mean differences were considered significant at $P < 0.05$.

Results and discussion

Eggshell microbial counts

Results for surface eggshell microbial loads are presented in Table 15. The sanitized treatment had the lowest surface APC (1.76 $\log_{10}$ cfu/egg) compared to both the control (5.09 $\log_{10}$ cfu/egg; $P < 0.001$) and washed (3.03 $\log_{10}$ cfu/egg; $P < 0.001$) treatments. These results are similar to previous studies that evaluated the effects of the H_2O_2/UV AOP method on chicken eggs where APC and *Salmonella* were reduced compared to untreated eggs (Wells, et al., 2011b; Fuchs, 2013; Al-Ajeeli, et al., 2016; Rehkopf, et al., 2017). Additionally, previous results from

chapter IV where the effects of the H_2O_2/UV AOP method on turkey hatching eggs also indicated that the sanitized treatment was reduced for surface eggshell microbial loads compared to the control and washed treatments.

As previously stated, to prevent potential pathogenic contamination to a flock, it is important to decrease eggshell microbial loads prior to incubation. Potential pathogenic microorganisms have shown to be present on the surface of the eggshell, with *E. coli* being the primary pathogen of concern in the turkey industry (Montgomery, et al., 1999). Excessive eggshell microbial contamination could lead to increased embryonic mortality and decreased hatchability with the potential for disease outbreak at the grow-out farm (Sacco, et al., 1988).

Table 15. Turkey eggshell surface aerobic plate counts (log_{10} cfu/ml ± SE)[1]

Treatment	Average
Control	5.09 ± 0.18^{a}
Washed	3.03 ± 0.23^{b}
Sanitized	1.76 ± 0.28^{c}

[a-c] Means within a column having different superscripts are significantly different ($P < 0.05$).
[1]n = 10 eggs per treatment.

Embryonic mortality and hatchability

Eggs removed during candling were broken out on day 26 prior to setting in hatchers, and unhatched eggs on day 28. Eggs were categorized as infertile, early dead, late dead, pipped, or rotten. Embryonic mortality and hatchability results are presented in Table 16. No significant differences were observed for embryonic mortality, pipped, rotten, or hatchability. Although surface eggshell microbial loads were significantly reduced, the use of the H_2O_2/UV AOP method did not improve embryonic mortality or hatchability when compared to the control and washed treatments. The lack of significant differences in this study might be due to the loss of

one replicate from the control treatment due to the previously mentioned incubator malfunction. The loss of the control incubator replicate likely reduced the statistical power of the ANOVA such that differences between treatments for hatchability and embryonic mortality were not found.

A study conducted by Wells, et al. (2011b) using chicken hatching eggs indicated that the application of the H_2O_2/UV AOP method was effective at surface eggshell microbial load reduction when compared to untreated control eggs, but also found no improvement of hatchability by application of the H_2O_2/UV AOP method. Additionally, a previous study consisting of 2 trials was conducted where the effects of UV radiation of turkey eggs was evaluated for microbial load reduction, embryonic mortality, and hatchability (Russo, 2001). Results from that study indicated that surface eggshell microbial loads were reduced, and embryonic mortality was not impacted compared to untreated turkey eggs for both trials. However, hatchability of UV radiated turkey eggs was improved in 1 trial only, with no differences in hatchability on the other trial.

Table 16. Turkey egg embryonic mortality and hatchability[1]

Treatment	Early dead[2]	Late dead[3]	Pipped	Total embryonic mortality	Rotten	Hatch of fertile
	% ± SE					
Control	3.87 ± 0.19	8.03 ± 2.37	10.60 ± 0.28	22.50 ± 2.46	0.00 ± 0.00	77.52 ± 2.46
Washed	3.48 ± 0.15	6.61 ± 0.63	9.23 ± 0.98	19.32 ± 0.75	0.00 ± 0.00	80.70 ± 0.75
Sanitized	3.67 ± 1.09	9.46 ± 2.64	9.96 ± 2.01	23.09 ± 5.08	0.17 ± 0.17	76.61 ± 4.99

[1]n = 2 incubators for the control treatment and 3 incubators for the washed and sanitized treatments..
[2]Early dead = day 1 to 14 of embryogenesis.
[3]Late dead =day 15 to 28 of embryogenesis.

Moisture loss and poult weight

Moisture loss for day 1 to 25 of incubation is shown in Table 17. No significant differences were observed between the treatments for egg moisture loss or poult weight. However, it was noted that the sanitized treatment moisture loss was slighter more than the control and washed treatments. This could have impacted average poult weight. Similar results were observed in previous studies that used the H_2O_2/UV AOP method on chicken hatching eggs which showed no differences in either moisture loss or chick weight (Wells, et al., 2011b). Duck hatching egg moisture loss and duckling weight results from chapter III are also similar further indicating a trend on the sanitized treatment having numerically greater moister loss than the control and washed treatments. The numerical increases of moisture loss in both chapter III and this experiment might indicate that slight alteration of eggshell porosity occurred with the H_2O_2/UV AOP method on duck and turkey hatching eggs.

Table 17: Turkey egg moisture loss[1] and poult weight at hatch[2]

Treatment	Moisture loss (%)	Poult weight (g)
Control	10.51 ± 0.10	65.39 ± 0.83
Washed	9.80 ± 0.54	66.00 ± 0.50
Sanitized	10.74 ± 0.31	63.90 ± 1.84

[1] Moisture loss calculated based on the difference of egg weight on day 0 and 25 of incubation.
[2] n = 3 incubators per washed and sanitized treatment, and 2 incubators for the control treatment.

Poult quality

Poult quality assessment is presented in Table 18. No differences were observed between treatments for any quality parameter evaluated in this study. While not statistically different, the sanitized treatment had a numerically lower percent of naval tags compared to the control treatment. Similar results were demonstrated in chapter III where the overall naval tag

percentage was significantly lower in sanitized treated duck eggs compared to untreated control eggs. Additionally, a study conducted by Fuchs (2013) indicated a similar trend to this study. When chicken hatching eggs were sanitized using the H_2O_2/UV AOP method, there was a numerical decrease in naval tags compared to untreated control eggs. That study also indicated a decrease in surface eggshell microbial loads. Perhaps the decrease in microbial loads on the eggshell surface by the H_2O_2/UV AOP method could attribute to the numerical decrease in naval tags obtained for poult quality.

Table 18. Poult quality assessment at hatch

Treatment	Naval tags	Dirty feathers	Cull poults	Good Poults[1]
Control	50.24 ± 1.99	0.67 ± 0.03	0.67 ± 0.03	48.42 ± 1.93
Washed	35.37 ± 0.78	0.44 ± 0.44	1.50 ± 0.42	62.69 ± 0.82
Sanitized	39.75 ± 0.89	0.89 ± 0.15	0.69 ± 0.43	58.67 ± 0.89

[1] Good poults calculated as a percentage of hatched poults without naval tags, dirty feathers, or culled.

Conclusions

This study demonstrated that the H_2O_2/UV AOP method applied to turkey hatching eggs was effective at significantly reducing surface microbial loads compared to the control and washed treatments. However, the H_2O_2/UV AOP method did not significantly impact embryonic mortality, hatchability, or poult quality. Nevertheless, a trend could be noted for poult quality and moisture loss in this experiment. Moisture loss was numerically greater for the H_2O_2/UV AOP treated eggs compared to the untreated control eggs. Additionally, naval tags were numerically lower in sanitized eggs compared to control eggs. Previous studies using the H_2O_2/UV AOP on chicken and duck eggs have also demonstrated these trends. To further analyze the previously mentioned trends and effects of the H_2O_2/UV AOP on turkey hatching

eggs, additional research should be conducted where multiple trials are performed. In addition, to further understand the differences between egg components of chicken, turkey, and duck species, differences in cuticle structure and protein concentrations should be assessed. This information could be valuable to optimize an effective egg sanitization method for each particular poultry species.

CHAPTER VI

CONCLUSIONS

Turkey and duck eggs are highly soiled in nature and typically have high microbial loads. It is important to reduce microbial contamination of hatching eggs prior to incubation, especially with the growing demand for antibiotic-free poultry production. Eggshell microbial contamination could lead to decreased hatchability and poor hatchling quality. Contamination of eggs with pathogens could follow the flock throughout production and lead to disease in the birds and food safety hazards for consumers (Berrang, 1999; Coufal, et al., 2003). To reduce such contamination on eggshells, the commercial turkey and duck breeder industries typically wash hatching eggs (Patterson, et al., 1990). Therefore, implementing an effective and commercially feasible method of hatching egg sanitization could increase flock survivability and have economic benefits (Sheldon and Brake, 1991; Berrang, et al., 1997; Spickler, et al., 2011). The effects of the H_2O_2/UV AOP method of egg sanitization have not previously been evaluated for turkey and duck hatching eggs. Thus, the primary objective of this study was to evaluate the use of the H_2O_2/UV AOP method of egg sanitization on turkey and duck hatching eggs.

Data from laboratory experiments demonstrated that both the H_2O_2/UV AOP treated and the standard washed eggs had lower surface APC compared to untreated control eggs. While the results indicated that the washing process was effective at reducing APC on the surface of eggs, the subsurface APC of the washed eggs was similar to untreated control eggs. However, it was found that the H_2O_2/UV AOP method was effective at reducing subsurface APC compared to both the washed and untreated control eggs for both turkey and duck eggs. Furthermore, the use of selective and differential media for enumeration of various microorganisms from turkey and duck eggs demonstrated that the H_2O_2/UV AOP method was effective at reducing total

coliforms, fungi and aciduric microorganisms, *Enterococcus* spp. and *Staphylococci* compared to untreated control eggs. Additional reductions of *Escherichia coli* and yeast and mold counts were demonstrated for turkey eggs treated with the H_2O_2/UV AOP method compared to untreated control eggs. Prior to the present study, previous research with the H_2O_2/UV AOP method evaluated APC and inoculated *Salmonella* reductions only. The present study demonstrated that the H_2O_2/UV AOP method was effective at reducing various naturally-occurring eggshell surface and subsurface bacteria and fungi that could be potential pathogens of concern for the poultry industry.

Following successful laboratory trials, the H_2O_2/UV AOP prototype egg sanitizer was transported to a commercial duck hatchery for field trials. Experiment 2, Trial 1 demonstrated that the H_2O_2/UV AOP method and the washed treatment resulted in similar surface eggshell microbial counts. However, in Experiment 2, Trial 2 the combination of the washing process followed by the H_2O_2/UV AOP method demonstrated additional reductions of surface eggshell microbial counts compared to the washing process or the H_2O_2/UV AOP method alone.

Similar to laboratory experiments, data from field evaluations demonstrated that the H_2O_2/UV AOP method had lower subsurface APC than both the washed and untreated control eggs. Raw data from the 4 trials in Chapter IV are presented in Tables 19 to 22 and provide a summary of the subsurface APC. In addition, a follow-up field trial not previously reported in this study is presented on Table 23. In general, the washing process did not result in subsurface APC reductions compared to untreated control eggs. However, utilization of the H_2O_2/UV AOP method showed lower subsurface APC counts compared to the washing process in all trials except Experiment 2, Trial 2. However, in that trial, the combination of the washing process followed by the H_2O_2/UV AOP treatment had lower subsurface eggshell APC than the washed

eggs alone. The results appear to indicate that the H_2O_2/UV AOP method has the ability to impact microbes below the surface of the eggshell. The common commercial washing process has shown to reduce microbial loads on the surface of the egg, but not in the subsurface. The lack of subsurface APC reductions could be due to the washing process mobilizing microorganisms into the pores of the eggshell. Additionally, failure of the detergents and sanitizers in the washing solution to interact with pre-existing microorganisms inside the pores and shell membranes of the egg could also explain the washing process subsurface APC results.

Table 19. Experiment 1, Trial 1 raw data of duck egg subsurface APC (log_{10} cfu/egg)

Egg number	Control	Washed	Sanitized
1	5.03	2.81	3.09
2	3.95	3.40	2.30
3	3.90	5.94	3.02
4	3.60	2.72	3.72
5	6.92	3.68	3.54
6	4.70	5.76	3.35
7	4.43	6.04	2.97
8	3.18	4.99	2.51
9	3.70	5.22	2.35
10	3.94	2.51	4.05
Average	4.34	4.31	3.09

Table 20. Experiment 1, Trial 2 raw data of duck egg subsurface APC (log_{10} cfu/egg)

Egg number	Control	Washed	Sanitized
1	4.35	2.51	2.10
2	2.99	2.24	2.89
3	3.57	2.10	1.10
4	2.63	3.15	1.10
5	3.28	1.70	1.10
6	1.40	1.70	1.10
7	2.18	2.00	1.10
8	3.01	2.10	1.10
9	2.24	1.10	2.35
10	2.57	5.51	1.10
Average	2.82	2.41	1.50

Table 21. Experiment 2, Trial 1 raw data of duck egg subsurface APC (log_{10} cfu/egg)

Egg number	Control	Washed	Sanitized once	Sanitized twice
1	3.70	TNTC[1]	TNTC[1]	4.13
2	4.34	TNTC[1]	4.39	3.18
3	4.40	TNTC[1]	2.95	4.17
4	4.23	3.40	4.59	2.51
5	3.40	2.94	1.10	2.30
6	4.24	2.68	3.74	4.36
7	TNTC[1]	2.98	2.60	TNTC[1]
8	4.07	4.60	TNTC[1]	4.39
9	4.75	NA[2]	4.26	4.65
10	3.40	TNTC[1]	3.90	4.33

[1]Too numerous to count.
[2]Egg was cracked upon arrival and therefore subsurface microbial enumeration procedures were not able to be performed.

Table 22. Experiment 2, Trial 2 raw data of duck egg subsurface APC (log_{10} cfu/egg).

Egg number	Washed	Sanitized	Washed + sanitized
1	4.80	4.05	4.35
2	1.70	3.44	1.88
3	2.18	4.08	1.10
4	4.25	3.39	1.10
5	1.10	5.65	1.10
6	1.10	1.10	1.10
7	2.10	4.65	3.72
8	3.04	1.10	1.10
9	5.27	2.97	5.33
10	2.10	4.04	3.04
Average	2.76	3.45	2.38

Table 23. Follow-up trial (not previously reported) raw data of duck egg subsurface APC (log_{10} cfu/egg)[1]

Egg number	Washed	Washed + sanitized
1	4.70	3.16
2	4.74	1.70
3	4.49	2.80
4	7.04	3.81
5	6.38	5.17
6	1.10	4.27
7	3.04	3.27
8	5.57	2.85
9	2.30	3.98
10	3.65	2.70
Average	4.30	3.37

[1]Trial was not previously reported in this study. All egg treatment was performed at a commercial hatchery using similar techniques as described in Chapter IV for subsurface APC.

Results for duck embryonic mortality demonstrated that the eggs treated with the H_2O_2/UV AOP method had the lowest total embryonic mortality compared to the washed and untreated control eggs. Furthermore, this study indicated that duck hatchability was improved using the H_2O_2/UV AOP method by approximately 13% compared to the untreated control eggs and 9% compared to the washed eggs. In contrast, embryonic mortality and hatchability results for turkey eggs indicated no differences between the H_2O_2/UV AOP treated, washed and untreated control eggs. Variability in hatchability results between turkeys and ducks could indicate differences in susceptibility to eggshell microbial invasion. Differences in egg structure between turkey and duck eggs might be a factor that could influence the ability of microorganisms to penetrate the eggshell. As previously mentioned, the inner shell membrane is an important innate defense component against microbial contamination. Duck eggs have the thinnest shell membrane compared to chicken and turkey eggs, with similar inner shell membrane weight as chicken eggs. Additionally, turkey and duck eggs have lower relative shell

membrane density compared to chicken eggs. The shell membranes are an important physical barrier against penetration of bacteria. Lower shell membrane density implies the shell membrane fibers are less dense, and thus may offer less protection against inward microbial invasion. Overall, duck eggs could be more susceptible to eggshell microbial contamination compared to chicken and turkey eggs due to their differences in egg structure discussed in Chapter II and hatchability results obtained in this study.

Another explanation for the differences in hatchability obtained in this study between turkeys and ducks could be that turkeys are more closely related phylogenetically to chickens. Previous research with chicken hatching eggs that evaluated the effectiveness of the H_2O_2/UV AOP method indicated that hatchability was not impacted by treatment of chicken hatching eggs with 6 repetitions of the H_2O_2/UV AOP method compared to untreated control eggs (Wells, et al., 2011b). However, a study conducted by Fuchs (2013) on chicken hatching eggs evaluating the effectiveness of the H_2O_2/UV AOP method indicated that hatchability was improved by 2 repetition of the H_2O_2/UV AOP method compared to untreated control eggs. Treatment of turkey hatching eggs by 4 repetitions (2 passes through prototype egg sanitizer) of the H_2O_2/UV AOP method in this study might not be optimal for hatchability in the same manner as for duck hatching eggs. Additional research should be conducted on turkey hatching eggs to evaluate 2 repetitions (one pass through prototype egg sanitizer) of the H_2O_2/UV AOP method for microbial enumeration, hatchability, and poult quality.

Hatchling quality results from this study were similar between turkey and duck species. Duckling quality data indicated that the H_2O_2/UV AOP treated eggs resulted in a greater percent of ducklings with no defects compared to the control eggs. Poult quality results demonstrated no statistical differences between treatments. However, it was observed that the average number of

naval tags for poults and ducklings from eggs treated with the H_2O_2/UV AOP method was numerically fewer compared to control eggs. The numerical decrease of naval tags might be associated with the eggshell microbial load reductions discussed previously. Similar results were observed in a previous study conducted by Fuchs (2013) with chicken eggs treated with the H_2O_2/UV AOP method. Results from that study indicated no statistical differences in naval tags between sanitized and untreated control eggs. An increase in naval tags is an issue associated with economic loss in the poultry industry. Increased naval tags are a hatchling quality concern because it could lead to infection of the naval (omphalitis) which could lead to reduced weight gain or bird mortality.

Further research should be conducted to evaluate the effectiveness of the H_2O_2/UV AOP method as an additional disinfection step to a commercial hatching egg washing process due to the increased surface APC reductions observed in this study and the ease of commercial implementation. Microbial loads, hatchability, and hatchling quality should be further assessed with turkey and duck hatching eggs which are typically washed prior to incubation. To further understand the differences in hatchability between chicken, turkey and duck species, the differences in egg components (specifically cuticle structure) and microbial invasion susceptibility should be analyzed. Information on the differences in eggshell innate defenses could aid in development of an optimal egg sanitization method that is best suited for each species.

Proper hatching egg sanitization is an important step in poultry production that could ultimately have economic implications. Eggshell contamination could lead to a decrease in hatchability, a decrease in hatchling quality, and continuation of infection at the grow-out farm. Additionally, contamination by bacteria such as *Salmonella*, *Escherichia coli* and

Staphylococcus aureus could pose foodborne illness concerns for consumers. The removal of antibiotics in the poultry production chain may increase the probability of flock infection and disease. However, implementing an effective and commercially feasible method of hatching egg sanitization is an important preventative step to decrease microbial contamination. Hatching egg sanitization prior to incubation could have economic gain and public health significance.

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